JOURNAL OF AGRICULTURAL RESEARCH

VOL. 58 WASHINGTON, D. C., MARCH 1, 1939 No. 5

THE USE OF THE ANALYSIS OF COVARIANCE AND ITS LIMITATION IN THE ADJUSTMENT OF YIELDS BASED UPON STAND IRREGULARITIES [1]

By CHARLES H. MAHONEY, formerly *research associate in horticulture, Michigan Agricultural Experiment Station*, and W. D. BATEN, *research associate in statistics, Michigan Agricultural Experiment Station*

INTRODUCTION

In the course of investigations dealing with the breeding of vegetable crops, where progenies were tested in comparision with commercial strains, more perfect stands were very frequently obtained from seed of the latter. This was particularly true for hand-pollinated hybrid sweet corns. Even though replanting can be used, it is often difficult to obtain perfect stands with such widely spaced transplanted crops as tomatoes and muskmelons. Methods were investigated, therefore, for correcting for stand irregularities on plots which had been so designed that statistical interpretation could be made by the analysis of variance.

EFFICIENCY GAINED BY ADJUSTING YIELDS

The influence of stand on yield has been investigated by numerous workers. Bryan (*4*)[2] working with corn has shown that stand influenced yield more significantly than border effect, and he states that "adjustment for perfect hill stands reduced the number of hills required for any degree of precisions by 18.9 percent." Matthews (*7*) and Gillis (*6*) have shown that wider spacings have reduced yields of snap beans and lima beans. Beattie and Boswell (*2*) found that machine seeding of row crops did not insure perfect stands, and they found a high correlation between stand and yield of both carrots and onions grown on muck soil. The influence of stand on yield has not been thoroughly investigated for all vegetable crops, and this correlation must be established before any degree of precision will be attained in correcting yield on the basis of stand. Beattie, Boswell, and Batten (*3*) have shown the absence of correlation between stand and yield of peanuts.

The methods of adjusting yield used in this paper are not original and have been presented by various statistical workers, but it is hoped that presentation of examples of the use of the regression coefficient obtained from variance and covariance as a means of adjusting yields for irregularities of stand will be of value to other workers with vegetable crops for more adequately interpreting the results of their field experiments.

The data from randomized plots of 26 hybrid strains and 1 open-pollinated strain of yellow sweet corn will be used to illustrate one

[1] Received for publication May 6, 1938. Journal paper No. 326 of the Michigan Agricultural Experiment Station.

[2] Italic numbers in parentheses refer to Literature Cited, p. 328.

case where the efficiency of the experiment has been increased by adjustment of yields on the basis of stand. The yield-record sheets from 5 of the 108 plots were lost, and the method of estimation for missing plots, as suggested by Yates (*11*) and Allan and Wishart (*1*), has been used to determine estimates of these plots. This method is (*Number of treatments* × *total of treatments*) + (*Number of replications* × *total of replications*) − *total of all plots* divided by (number treated − 1) × (number replicated − 1)[3]

The data on yields and number of plants for the 108 plots are presented in table 1.

TABLE 1.—*Yield of marketable ears and numbers of plants (stand) of 108 plots with mean yields and stands for 27 strains of yellow sweet corn grown in 1937 (unadjusted)*

Item	Yield and stand for strain No.—													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Replication 1:														
Yield (*Y*)	56	31	21	23	50	60	46	58	31	42	31	61	47	56
Stand (*X*)	77	68	61	83	70	84	72	81	79	71	61	73	58	81
Replication 2:														
Yield (*Y*)	64	29	32	20	59	50	50	50	39	53	36	55	65	65
Stand (*X*)	80	76	72	88	89	92	82	83	74	89	68	68	64	90
Replication 3:														
Yield (*Y*)	56	30	24	18	60	47	55	62	48	60	34	62	62	58
Stand (*X*)	74	83	82	78	78	78	84	96	75	89	81	85	71	102
Replication 4:														
Yield (*Y*)	36	32	[1] 24	[1] 19	39	[1] 50	55	55	40	49	34	48	59	62
Stand (*X*)	57	61	73	78	52	88	97	77	71	58	67	77	67	97
Strain yield totals	212	122	101	80	208	207	206	225	158	204	135	226	233	241
Strain stand totals	288	288	288	327	289	342	335	337	299	307	277	303	260	370
Mean yield	53.0	30.5	25.3	20.0	52.0	51.8	51.5	56.3	39.5	51.0	33.8	56.5	58.3	60.3
Mean stand	72.0	72.0	72.0	81.8	72.3	85.5	83.8	84.3	74.8	76.8	69.3	75.8	65.0	92.5

Item	Yield and stand for strain No.—													Replication totals	
	15	16	17	18	19	20	21	22	23	24	25	26	43	Yield	Stand
Replication 1:															
Yield (*Y*)	52	54	46	24	55	57	46	55	39	67	64	63	45	1,280	.....
Stand (*X*)	94	72	63	34	80	73	78	75	62	74	96	81	64	.....	1,968
Replication 2:															
Field (*Y*)	73	50	55	32	57	61	55	51	48	79	57	72	50	1,407	.....
Stand (*X*)	98	82	62	62	81	73	84	64	76	88	80	98	62	.....	2,131
Replication 3:															
Yield (*Y*)	74	65	[1] 56	50	50	37	65	52	50	66	66	74	56	1,437	.....
Stand (*X*)	110	97	60	60	94	81	80	84	77	90	92	103	78	.....	2,271
Replication 4:															
Yield (*Y*)	64	64	51	26	[1] 52	41	62	54	51	53	60	73	53	1,309	.....
Stand (*X*)	92	82	60	24	77	68	73	76	66	85	87	87	71	.....	1,975
Strain yield totals	263	233	208	132	214	196	228	212	188	265	247	282	204	5,430	.....
Strain stand totals	394	333	262	180	332	295	315	299	287	337	355	367	278	.....	8,345
Mean yield	65.8	58.3	52.0	33.0	53.5	49.0	57.0	53.0	47.0	66.3	61.8	70.5	51.0	50.1	.....
Mean stand	98.5	83.3	65.8	45.0	83.0	73.8	78.8	74.8	71.8	84.3	88.8	91.8	69.5	.....	77.3

[1] Estimation of missing data.

The analysis of variance and covariance for the yields of marketable ears and for stand (number of plants per plot) is given in tables 2 and 3.

[3] This formula was used once for estimating each missing plot yield.

TABLE 2.—*Analysis of variance and covariance for 1937 hybrid sweet-corn trials for unadjusted data in table 1*

Source	Degree of freedom	Yield, Y		Stand, X		Covariance, XY	
		Sum of squares	Variance	Sum of squares	Variance	Sum of products	r=correlation coefficient
Total	[1] 102 107	20,174		18,475		9 766	
Replications	3	646	215.33	2,315	[3] 771.7	1,178	[3] 0.963
Strains	26	16,206	[2] 623.31	11,752	[2] 452.0	7,326	[3] .531
Error	[1] 73 78	3,322	45.51	4,408	56.5	1,262	[3] .330
		Strain F=13.70 [4]		Strain F=8.00		b (regression coefficient)=0.2863, obtained from (xy) and $(x)^2$ for error	
		[3] 1.67 [2] 2.07		[3] 1.67 [2] 2.07			
Standard error of experiment		6.75		7.52			

[1] Degrees of freedom for yield, because of estimating 5 missing yields is 102. Degrees of freedom for stand is 107.
[2] 1-percent point. [3] 5-percent point.
[4] See table 3 for "best" value of F for testing significance between strain means.

TABLE 3.—*Analysis for testing significance between strain-yield means because of missing plots* [1]

Source	Degrees of freedom	SS (sum of squares)	Variance
Totals [2]	102	18,444.41	45.51
Error [2]	73	3,322.00	
Block+treatments	29	15,122.41	
Blocks only [4]	3	498.83	
Difference	26	14,623.58	562.45

$$F=\frac{562.45}{45.51}=12.4.$$

[1] See Yates (*11*).
[2] From table 2.
[3] Obtained from original yields not including estimates of missing yields.
[4] Obtained from block totals without the estimates of missing yields.

It is evident that the correlation coefficients between stand and yield in table 2 are significant. This was to be expected as seed is often a limiting factor when planting hand-pollinated single crosses and invariably more seed is planted and a better stand obtained with established commercial hybrids. That there is a definite correlation between stand and yield is further borne out by the highly significant correlation coefficient of the error components shown in the last column of table 2. Since this is true, it would follow that adjustment of yields on the basis of stand might logically change the mean yields and ranks of some of the hybrids. If the regression coefficient is computed, it is possible by the use of the following equation, suggested by Miles and Bryan (*8*), to adjust the yields of marketable ears for each of the 108 plots:

$$Y'=Y-b\,(X-Mx) \text{ where}$$

Y'=adjusted yield
Y=actual individual yields
X=actual individual stands
Mx=mean of all stands
b=regression coefficient, obtained from $\Sigma(xy)$ and $\Sigma(x)^2$ for error.

The adjusted yields, carried out to two decimal places derived by the foregoing equation, are given in table 4

TABLE 4.—*Yield of marketable ears of 108 plots of 27 strains of sweet corn adjusted on the basis of stands in table 1*

Item	Yield of Strain No. —													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Replication 1	56.09	33.66	25.66	21.37	52.09	58.08	47.52	56.94	30.51	43.80	35.66	62.23	52.52	54.94
2	63.23	29.37	33.52	16.94	55.65	45.80	48.66	48.37	39.94	49.65	38.66	57.66	68.80	61.37
3	56.94	28.37	22.66	17.80	59.80	46.80	53.08	56.65	48.66	56.65	32.94	59.80	63.80	50.94
4	41.81	36.66	25.23	18.80	46.24	46.94	49.37	55.09	41.80	54.52	36.95	48.09	61.95	56.37
Strain means	54.52	32.02	26.77	18.73	53.45	49.41	49.66	54.26	40.23	51.16	36.05	56.95	61.77	55.91

Item	Yield of Strain No. —													Replication	
	15	16	17	18	19	20	21	22	23	24	25	26	43	Total	Sums of squares
Replication 1	47.22	55.52	50.09	36.38	54.23	58.23	45.80	55.66	42.52	67.94	58.65	61.94	48.80	1,314.05	67,505.06
2	67.08	48.66	59.38	36.38	55.94	62.23	53.08	54.80	47.51	75.94	56.23	66.08	53.52	1,394.45	76,508.76
3	64.65	59.37	58.37	54.95	45.22	35.94	64.23	50.08	50.09	62.37	61.80	66.65	55.80	1,384.41	75,448.89
4	59.80	62.66	53.37	41.24	52.09	43.66	63.23	54.37	54.23	50.80	57.23	70.80	54.80	1,338.10	69,746.42
Strain means	59.69	56.55	55.30	42.24	51.87	50.01	56.59	53.73	48.59	64.26	58.48	66.37	53.23	(5,431.01) [1] 50.29	289,209.13

[1] General mean.

After the adjusted individual plot yields were determined an analysis of variance for adjusted yields was then carried out; this is shown in table 5, and an analysis for securing a test of significance between adjusted strain yield means is presented in table 6.

TABLE 5.—*Analysis of variance of yields of marketable ears for 1937 hybrid sweet corns (individual plot yields adjusted)*

Source	Degrees of freedom	Sum of squares	Variance [1]	Standard error
Total	[1] 101	16,098		
Replication	3	161	53.7	
Strain	26	12,977	[2] 499.1	
Error	72	2,960	41.1	
			$F=12.1$	
			[3] 1.67	
			[4] 2.07	
				6.41

[1] 1 degree of freedom less than given in table 2 as 1 degree was lost in the use of the regression coefficient b.
[2] Significant to 1-percent level.
[3] 5-percent point.
[4] 1-percent point.

TABLE 6.—*Analysis for securing a test of significance between adjusted strain yields*

Source of variation	Degrees of freedom	Sums of squares and products			Errors of estimate for yields		
		Sx^2	$S(xy)$	Sy^2	Sum of squares	Degrees of freedom	Variance
Strain [1]	26	11,752	7,326	16,206			
Error [1]	73	4,408	1,262	3,322	2,960.69	[2] 72	[3] 41.1
Strain plus error	99	16,160	8,588	19,528	[3] 14,964.03	98	
Strain					[4] 12,003.34	26	461.7

$$F=\frac{461.7}{41.1}=11.2$$

[1] These values came from table 2.
[2] Because 1 degree of freedom is used up by using b.
[3] Found by employing the formula $Sy^2-\frac{S^2(xy)}{Sx^2}$=sum of squares of errors of estimate. The quantity Sy^2 is the sum of squares of y for strain + error, $S^2(xy)$ is the square of the sum of products for strain + error, and Sx^2 is the sum of squares of x for strain + error. This can also be found by using the formula $S(y^2)-2bS(xy)+b^2S(x^2)$, where b is found from the line strain + error, and the sums of squares and sum of products are taken from the strain + error line in the above table.
[4] Found by the subtraction [14,964.03−2,960.69=12,003.34].

It can be seen readily by comparing tables 2 and 5 that the variances have been reduced throughout, but the effectiveness of the method is evident in the reduction of the standard error of the experiment. Before adjustment 4 hybrids were significantly higher, 5 significantly lower, and 17 showed no significant differences, with reference to open-pollinated Golden Bantam. After the yield had been adjusted on stand, 2 hybrids were higher, 6 lower, and 18 not significantly higher or lower than open-pollinated Golden Bantam.[4]

As a result of adjustment two hybrids which previously had appeared significantly higher than the check, open-pollinated Golden Bantam, were found to be not significant. There was no change in the rank of the two highest producing varieties. The ranks of the first seven high-producing strains before and after adjustment are presented in table 7.

[4] The standard error of the difference between two adjusted yield means y_p and y_q is

$$s\sqrt{\frac{2}{n}+(\bar{x}_p-\bar{x}_q)^2/\Sigma(x-\bar{x})^2},$$

where s is the square root of the variance for error, $\Sigma(x-\bar{x})^2$ is sum of squares for stand in same line with error, n the number of replications, $\bar{x}_p$ the mean of stand for p^{th} variety, $\bar{x}_q$ the mean of stand for the q^{th} variety This method was suggested by Goulden.[5]

[5] GOULDEN, C. H. METHODS OF STATISTICAL ANALYSIS. Rev. ed., 209 pp. Minneapolis. 1937. [Mimeographed.]

TABLE 7.—*Ranking before and after adjusting for stand for the seven highest producing strains*

Variety key No.	Ears per plot before adjustment	Rank	Ears per plot after adjustment	New rank	Variety key No.	Ears per plot before adjustment	Rank	Ears per plot after adjustment	New rank
	Number		*Number*			*Number*		*Number*	
26	70.5	1	66.4	1	25	61.8	4	58.5	5
24	66.3	2	64.3	2	16	58.3	[1] 5	56.6	[1] 6
13	58.3	[1] 5	61.8	3	21	57.0	7	56.6	[1] 6
15	65.8	3	59.7	4					

[1] Tied.

It might be of interest from the canners' standpoint to point out that 13 hybrids produced a significantly higher yield of green unhusked corn per acre, and only 8 which were significantly lower than open-pollinated Golden Bantam. Likewise 13 hybrids were significantly higher and only 3 significantly lower than Golden Bantam for yield of husked green corn per acre.

The above example illustrating methods for correcting the yields of individual plots and setting up the analysis of variance pertaining to adjusted yields has been presented as a background for a much simpler and much less laborious method of obtaining the analysis of variance concerning adjusted data from the statistics presented in table 2. Using Sanders' (*9*) formula for finding sum of squares for adjusted yields, $b^2S(x)^2-2bS(xy)+S(y^2)$,[6]

Where $S(x)^2$ is the sum of squares for stand and $S(y)^2$ is the sum of squares for yield and $S(xy)$ is the sum of products, and substituting the statistics given in table 2 we get the following:

$(0.2863)^2\times18{,}475-[2(0.2863)\times9{,}766]+20{,}174=16{,}096.38$ for the new total sum of squares. This same formula is used to work out the new sum of squares for replications, strains, and error. The analysis of variance for adjusted yields worked out by this method is given in table 8.

TABLE 8.—*Analysis of variance for 1937 hybrid sweet corns adjusted for stand by short method* [1]

Source	Degrees of freedom	Sum of squares	Variance [1]	Source	Degrees of freedom	Sum of squares	Variance [1]
Total	101	16,096.38		Strain	26	12,974.44	[2] 499.0
Replication	3	161.22	53.7	Error	72	2,960.72	41.1

[1] See table 6 for test of significance between adjusted means. Standard error = 6.41.
[2] Significant to 1-percent level.

A comparison of tables 5 and 8 shows that the two analyses of variance are practically identical. The two total sums of squares differ by less than 2 units and sums of squares for replication are nearly identical. The sums of squares for strain and error differ by less than 3 units. It is necessary, in the calculation of plot yields by the first method, to work out the yields to three decimal places and to use four decimal places in the regression coefficient in order to make the two methods check. The adjusted means of the strains can then be obtained by use of the formula already given $Y'=\bar{Y}-b\ (X-Mx)$.[7]

[6] b is $S(xy)$ for error divided by $S(x)^2$ for error and is used to find the sum of squares in table 8.
[7] In this formula Y' now designates adjusted yield mean, $\bar{Y}$ the observed yield mean, and X the stand mean.

This short method originally given by Sanders (*9*) has later been described by Fisher (*5*), Snedecor (*10*), and Miles and Bryan (*8*).

SIGNIFICANCE BETWEEN STAND MEANS—NOTHING GAINED BY ADJUSTING

The advisability of adjusting seeded vegetable-plot yields on the basis of stand must be determined by the investigator. This can often be done by observation. If there is a fairly high variation due to soil heterogeneity and there are no appreciable differences in stand, usually nothing will be gained by adjustment. The analysis of variance for nine hydrids and one open-pollinated yellow sweet corn planted in 1935 and arranged in a 10 by 10 Latin square (table 9) showed a nonsignificant yield variance for strains, yet the strain variance for stand was significant. Would adjustment of yield on stand in this case disclose any significant strain means? The correlation coefficient r pertaining to error was found to be 0.247 (table 10), and with 71 degrees of freedom slightly exceeds Fisher's 5-percent point (*5*). The regression coefficient (b=0.442) was determined, and the new analysis of variance was made. This is presented in table 10 in comparison with the analysis of the original data.

TABLE 9.—*1935 sweet corn data on yield of marketable ears and stand of plants per plot for nine hybrids and one open-pollinated sweet corn planted in a Latin Square*

	1	2	3	4	5	6	7	8	9	10	Total of rows
Strain No.	C-4	C-7	C-3	C-6	C-8	C-10	C-5	C-1	C-9	C-2	
Yield	37	41	41	49	29	38	33	32	40	45	385
Stand	42	43	25	42	39	39	42	43	43	40	398
Strain No.	C-2	C-4	C-7	C-1	C-6	C-5	C-8	C-9	C-10	C-3	
Yield	43	40	48	40	46	31	41	34	30	34	387
Stand	42	47	45	46	39	48	46	45	39	31	428
Strain No.	C-1	C-8	C-9	C-5	C-4	C-2	C-3	C-7	C-6	C-10	
Yield	46	42	42	40	35	36	37	42	41	47	406
Stand	46	47	47	43	48	44	35	46	41	39	436
Strain No.	C-5	C-1	C-10	C-4	C-7	C-8	C-2	C-6	C-3	C-9	
Yield	49	46	40	36	45	45	43	46	38	47	435
Stand	41	44	44	42	45	43	45	48	37	44	433
Strain No.	C-10	C-5	C-4	C-9	C-2	C-3	C-6	C-8	C-7	C-1	
Yield	48	43	40	46	47	41	39	40	38	43	425
Stand	43	46	45	48	48	40	41	44	41	46	442
Strain No.	C-3	C-9	C-6	C-2	C-1	C-7	C-10	C-5	C-4	C-8	
Yield	42	48	40	40	50	51	35	53	42	50	451
Stand	36	41	42	41	45	46	36	46	48	46	427
Strain No.	C-9	C-3	C-1	C-10	C-5	C-6	C-7	C-2	C-8	C-4	
Yield	48	46	47	49	49	46	43	35	45	38	446
Stand	47	41	48	46	45	46	48	45	48	48	462
Strain No.	C-8	C-10	C-5	C-7	C-3	C-1	C-9	C-4	C-2	C-6	
Yield	44	48	44	52	48	48	45	47	42	44	462
Stand	48	46	47	46	48	47	48	47	45	45	467
Strain No.	C-7	C-6	C-2	C-8	C-10	C-9	C-4	C-3	C-1	C-5	
Yield	42	44	45	42	51	46	40	52	45	45	452
Stand	48	47	48	46	46	48	48	45	47	45	468
Strain No.	C-6	C-2	C-8	C-3	C-9	C-4	C-1	C-10	C-5	C-7	
Yield	50	41	47	51	48	44	45	40	39	55	460
Stand	48	45	47	41	48	48	47	47	47	47	465
Total of Stand	441	447	438	441	451	449	436	456	436	431	4426
Total of Yield	449	439	434	445	448	426	401	421	400	448	4311

	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	[1]C-10	
Total for strains Stand	459	443	379	463	450	439	455	454	459	425	4426
Total for strains Yield	442	417	430	399	426	445	457	425	444	426	4311
Mean for strains Stand	45.9	44.3	37.9	46.3	45.0	43.9	45.5	45.4	45.9	42.5	44.26
Mean for strains Yield	44.2	41.7	43.0	39.9	42.6	44.5	45.7	42.5	44.4	42.6	43.11
Adjusted yield means	43.5	41.7	45.8	39.0	42.3	44.5	45.2	42.0	43.7	43.4	

[1] Strain C-10 is open-pollinated Golden Bantam.

TABLE 10.—*Analysis of variance and covariance for 1935 sweet corn*

Source of variation	Degrees of freedom	X=stands		XY	Y=yields		Correlation coefficient	Errors of estimate of yield		
		Sum of squares	Variance	Sum of products	Sum of squares	Variance		Sum of squares	Degrees of freedom	Variance
Total	99	1,559.24	.....	699.14	2,813.79	.....	.....	[1]2,500.31	.....	.....
Between row means	9	470.04	52.23	472.14	750.09	83.34	[2]0.795	[1]275.84	.....	.....
Between column means	9	55.84	6.20	15.14	315.69	35.08	.114	[1]311.59	.....	.....
Between strain means	9	566.04	[3]62.89	5.34	246.89	27.43	.014	[1]246.84	9	.....
Error	72	467.32	6.49	206.52	1,501.12	20.85	[4].247	[1]1,409.99	71	19.86
Strain plus error	81	1,033.36	.....	211.86	1,748.01	.....	.....	1,704.57	80	.....
Strain	9	.....	.....	.....	.....	.....	.....	294.58	9	32.73
F for strain	.....	.....	[3]9.69	.....	.....	1.32	.....	.....	.....	1.65
b for error	.....	.....	.....	0.442	.....	.....	.....	.....	.....	.....
Standard error	.....	.....	.....	.....	.....	[5]4.57	.....	.....	.....	[5]4.46

[1] These values were found by using the formula where *b* is found from the sums of squares and sums of products. The first 4 values are not needed for finding a test for testing yield means for adjusted yields. These 4 will not be given in the remaining tables.
[2] Used in testing differences between yield means before adjustment.
[3] Significant at 1-percent level.
[4] Significant at 5-percent level.
[5] Used in testing differences between adjusted yield means.

The adjustment of yields on stand reduced the variance due to rows, and increased the variance due to strains, yet *F* for strain was not significant. The adjusted strain means, however, failed to show any that were either significantly higher or lower than open-pollinated Golden Bantam (C–10). The difference between the highest and lowest yielding strains was significant and there were three yields significantly larger than the lowest.

This is an example where the value of *F* indicated no significant differences between the means, yet the tests showed significant differences between the lowest yield means and the highest yield means. This was true before and after adjustment for stand. Nothing was gained here in adjusting for stand. The lowest mean yield was significantly different from four yield means before adjusting for stand.

NO SIGNIFICANCE BETWEEN STAND MEANS—NOTHING GAINED BY ADJUSTING

It is possible in randomized plots to obtain an insignificant treatment (fertilizer, strains, etc.) variance and yet obtain significant differences between some of the treatment means. This is likely in fertility plots where a large number of different fertilizers are applied and especially so if the units are doubled for each change in analysis or rate of application. Often there is no significant difference between the general mean and variety or treatment means, while there are significant differences between variety or treatment means. Horticulturally, we are more interested in comparing the treatments with a check (no treatment or grower's treatment) or with one another than with the general mean.

A good example of a case of this kind is the fertilizer analysis and rate of application experiment on tomatoes grown for canning at the Michigan station in 1937. Georgia plants were set in 4 randomized blocks, and 11 different treatments were used along with the check.

The original data on yield and stand are given in table 11, and the analysis of variance and covariance for yield of U. S. No. 1 and No. 2 tomatoes and stand is presented in table 12.

TABLE 11.—*Yield in pounds per plot of U. S. No. 1 and No. 2 grade combined and stand of plants per plot for 1937 tomato fertilizer experiment*

UNDER DIFFERENT TREATMENTS (T)

Item	Yield and stand of treatment—												Replication totals	
	T-1	T-2	T-3	T-4	T-5	T-6	T-7	T-8	T-9	T-10	T-11	T-12	Yield	Stand
Replication 1:														
Yield	177	161	254	217	145	214	155	176	133	262	190	113	2,197	
Stand	20	20	19	20	20	20	20	20	20	20	20	19		238
Replication 2:														
Yield	99	199	169	189	139	146	148	192	94	124	224	223	1,946	
Stand	20	20	16	20	20	20	20	20	20	19	19	20		234
Replication 3:														
Yield	78	133	102	132	242	176	172	213	118	182	181	193	1,922	
Stand	20	18	20	17	20	20	20	20	20	20	20	20		235
Replication 4:														
Yield	164	203	195	214	107	210	224	212	116	187	179	149	2,160	
Stand	20	20	20	20	20	18	20	19	20	19	20	20		236
Mean yield	129.5	174.0	180.0	188.0	158.2	186.5	174.8	198.2	115.2	188.7	193.5	169.5	[1] 171.4	
Mean stand	20.0	19.5	18.8	19.2	20.0	19.5	20.0	19.8	20.0	19.5	19.8	19.8		[1] 19.7
Adjusted yield means	127.2	175.5	186.9	191.8	155.9	188.0	172.5	197.4	112.9	190.2	192.4	168.7		

[1] General means.

TABLE 12.—*Analysis of variance and covariance for 1937 tomato fertilizer plots in table 11*

Source of variation	Degrees of freedom	X=Stands		XY	Y=Yields		Correlation coefficients	Errors of estimate of yield		
		Sum of squares	Variance	Sum of products	Sum of squares	Variance		Sum of squares	Degrees of freedom	Variance
Total	47	33		41	92,111					
Between replication means	3	1	0.333	53	5,063	1,688	0.745			
Between treatment means	11	6	.545	−211	28,763	2,615	−.508		11	
Error	33	26	.788	199	58,285	1,766	.162	56,761.9	32	1,774
Treatment plus error	44	32	1.333	−12	87,048			87,043.5	43	
Treatment	11							30,281.6	11	2,753
F for treatment			1.45			1.48				1.55
b for error					7.65					
Standard error						42.0				42.1

From an examination of table 12 it is evident that treatment F for stand is not significant and that probably there would be no significant differences between the means of the treatments. Since the check produced the lowest yield with one exception, there were two treatments which produced more than the ± 3.64 tons compared to the check necessary for significance, and these are beyond the 5-percent point. Three additional treatments had means which approached the ± 3.64-ton difference for significance. Even though there is no significant correlation between yield and stand, would it be advisable to adjust the yields on stand even though no appreciable variance is evident for stand? Further, would this increase the efficiency of the experiment and reduce the standard error so that the three means approaching significance would be significant? The necessary parts of the analysis of variance concerning data adjusted for stand, computed by the short method, already described are presented in columns 9, 10, and 11 of table 12.

The adjustment of yield on stand has increased the variance due to residual error. There is an increase in the treatment variance accompanied by a slight increase in the size of F for treatment, but F for treatment remains insignificant. However, the slight increase in the size of the standard error, the increases in adjusted yields for high-yielding treatments, and decreases in low-yielding treatments brought about significant differences between nine means. The mean yield of the check plot was decreased, and the mean yields of two of the treatments which approached significance before correction now show a difference which exceeds the necessary ± 3.59 tons for significance. There are now 4 treatments out of the 11 which exhibit significant differences over the check which may be attributed to fertilizer treatment. There are, however, no significant differences between any of the five high-yielding treatments.

The example given above demonstrates that it is possible to have significant differences between treatment means when F itself is not significant and also that adjustment of yield on stand as carried out here slightly increased the efficiency of the experiment. This often happens when there are such large differences between the extreme yields.

This is presented hereby as a statistical example and the experimental worker would hardly be justified in drawing any final conclusions from such data.

SIGNIFICANCE BETWEEN YIELD MEANS BEFORE ADJUSTING—NO SIGNIFICANCE AFTER ADJUSTING

Another example is presented where the yield treatment F is significant, the stand F approaches significance but, with one exception, none of the means show significant differences when compared with the check. These data are taken from the 1937 fertilizer-placement tests on Refugee beans grown for canning (table 13). The correlation coefficient for treatment is significant with a value equal to 0.913 but the r for error is 0.285 which for 17 degrees of freedom is not significant. The analyses of variances for the original data and the adjusted data are presented in table 14. The parts necessary for testing differences of adjusted yield means are given in columns 9, 10, and 11 of table 14.

TABLE 13.—*Yield in pounds per plot and stand for 1937 Refugee bean fertilizer-placement experiment*

Treatment	Yield and stand of plants per plot							Replication totals	
	B-1	B-2	B-3	B-4	B-5	B-6	B-7	Yield	Stand
Replication 1:									
Yield	11.6	12.2	3.9	10.3	7.9	11.3	9.3	66.5	
Stand	105	100	55	99	92	97	89		637
Replication 2:									
Yield	12.5	14.2	9.2	10.6	12.2	14.3	13.5	86.5	
Stand	100	103	108	103	97	91	100		702
Replication 3:									
Yield	11.6	12.1	8.2	12.5	12.6	11.4	10.5	78.9	
Stand	112	101	80	99	108	107	98		705
Replication 4:									
Yield	11.2	9.7	9.9	10.2	10.3	10.5	8.0	69.8	
Stand	109	97	87	91	108	108	106		706
Mean yield	11.7	12.1	7.8	10.9	10.8	11.9	10.3		
Adjusted yield means	12.0	12.2	8.4	10.9	10.9	12.1	10.3		

TABLE 14.—*Analysis of variance and covariance for 1937 Refugee bean fertilizer-placement experiment* [1]

Source of variation	Degree of freedom	X=Stands		(XY)	Y=Yield		Correlation coefficients	Errors of estimate of yield		
		Sum of squares	Variance	Sum of products	Sum of squares	Variance		Sum of squares	Degree of freedom	Variance
Total	27	3,495		394.25	126.3					
Between replication means	3	487	162.3	81.15	35.1	11.7	0.621			
Between treatment means	6	1,342	223.7	239.55	51.2	8.5	[1].914		6	
Error	18	1,666	92.6	73.55	40.0	2.2	.285	36.8	17	2.16
Treatment plus error	24	3,008		313.10	91.2			58.6	23	
Treatment								21.8	6	3.65
F for treatment			2.42			[2]3.86				1.69
b for error					0.0441					
Standard error						1.48				1.47

[1] Significant to 1-percent level. [2] Significant to 5-percent level.

That the variance for replication is significant before adjustment is clearly evident from table 14. Adjustment slightly reduced the residual error variance and the standard error, but it likewise reduced the treatment variance. The latter reduction was sufficient to reduce the significance of treatment *F*. There is an apparent reduction in efficiency if one considers only the lowered treatment variance. This was caused by correcting treatment No. 3 in which the fertilizer was placed below the seed, which very materially reduced the stand. This reduction in stand was very clearly evident in the field. If this treatment were corrected for stand and the yield computed from the regression equation, would this plot still be significantly lower? This was done; the mean for B–3 was not significantly less than the mean for the check (B–4). The mean of B–3 was significantly less than B–1, B–2, and B–6 after adjusting. It was significantly different from all the other means before adjusting. The variance for treatment yields is not significantly different from the variance for error after adjustment. Adjusting for stand reduced a significant *F* to a nonsignificant *F;* hence the differences in the beginning were due to differences in stand.

SUMMARY

The analysis of covariance between yield of marketable ears and stand of plants is presented for 26 hybrid strains and 1 open-pollinated strain of sweet corn. The yield data for five plots were lost, and a method of estimation for these missing plots is given. The correlation coefficient for error exceeds the 1-percent level, and an equation is given by which it is possible to adjust the yields on stand for each of the 108 plots.

A short method of deriving an analysis of variance for adjusted yields without the necessity of actually adjusting individual plot yields is presented. That this method is mathematically correct is substantiated by the derivation of essentially identical analyses of variance by both methods. The efficiency was increased by adjusting yields.

Another example is presented of maize planted in a 10 by 10 Latin square in which there was a significant residual correlation between yield and stand. The efficiency of the experiment, however, was not increased by adjustment of yield on stand in this case.

Data are presented for a fertility experiment with tomatoes grown from transplants. In this case treatment variance and the correlation coefficient between yield and stand were not significant, yet the efficiency gained in adjusting yield on stand is indicated when the adjusted treatment means are compared with the check (no treatment).

Additional data are presented, however, from a fertilizer placement test with Refugee beans. In this case the value of F for yields was not significant after adjusting for stand.

LITERATURE CITED

(1) Allan, F. E., and Wishart, J.
1930. A method of estimating the yield of a missing plot in field experimental work. Jour. Agr. Sci. [England] 20: [399]–406.

(2) Beattie, J. H., and Boswell, Victor R.
1936. Statistical studies of apparently uniform fields of carrots and onions on peat soils. Gartenbauwissenschaft 10: [279]–288.

(3) ——— Boswell, Victor R., and Batten, E. T.
1937. Plot and plant variation in Virginia peanuts. Amer. Soc. Hort. Sci. Proc. (1936) 33 (v. 34): 586–589.

(4) Bryan, Arthur A.
1933. Factors affecting experimental error in field plot tests with corn. Iowa Agr. Expt. Sta. Research Bull. 163, pp. [243]–280, illus.

(5) Fisher, R. A.
1934. Statistical methods for research workers. Ed. 5, rev. and enl., 319 pp., illus. Edinburgh and London.

(6) Gillis, M. C.
1929. The relation between rate of planting and yield in garden beans. Amer. Soc. Hort. Sci. Proc. (1928) 25: 80–86.

(7) Matthews, W. A.
1934. The influence of planting distances on the yield of snap and lima beans. Amer. Soc. Hort. Sci. Proc. (1933) 30: 567–570.

(8) Miles, L. G., and Bryan, W. W.
1937. The analysis of co-variance and its use in correcting for irregularities of stand in agricultural trials for yield. Roy. Soc. Queensland Proc. 48: 30–34.

(9) Sanders, H. G.
1930. A note on the value of uniformity trials for subsequent experiments. Jour. Agr. Sci. [England] 20: [63]–73.

(10) Snedecor, George W.
1937. Statistical methods applied to experiments in agriculture and biology. 341 pp., illus. Ames, Iowa.

(11) Yates, F.
1933. The analysis of replicated experiments when the field results are incomplete. Empire Jour. Expt. Agr. 1: 129–142.

A CRITICAL STUDY OF TECHNIQUE FOR MEASURING GRANULATION IN WHEAT MEAL[1]

By W. W. WORZELLA, *assistant in agronomy*, and G. H. CUTLER, *assistant chief in agronomy, Purdue University Agricultural Experiment Station*

INTRODUCTION

Meal granulation, or degree of particle fineness, is regarded as an important component of wheat quality and is of considerable interest to the miller, cereal chemist, geneticist, and plant breeder. A simple method, therefore, of accurately measuring meal granulation would greatly facilitate a more thorough study and analysis of this character. Although a number of studies dealing with flour and meal granulation have been reported, few data are available to show the steps in the procedure used in establishing a satisfactory technique. In a preliminary paper Cutler and Brinson [2] described a method for measuring granulation of whole wheat meal. Further studies have been made with a view to perfecting and standardizing this method. This paper, therefore, presents data emanating from a systematic study of the steps in the procedure evolved in establishing a suitable technique for measuring meal granulation. New equipment and the nature of meal fractions are also described.

REVIEW OF LITERATURE

Several reports have been published in which separations of flour or meal particles of varying degrees of fineness were obtained by sifting on various sizes of bolting cloth and sieves. Since most of these are concerned with the chemical composition and baking quality of the various-sized fractions, only the more pertinent papers will be discussed.

Balakshin [3] used a farinogranometer to determine the relative coarseness of semolina, wheat flour, and buckwheat flour. Ten grams of the product were placed on the nest of sieves and sifted by hand. The fractions collected on the sieves were placed in a series of compartments forming columns from which a coarseness curve was plotted. In addition, cumulative coarseness curves were made from which it was possible to determine the relative coarseness of products by comparing the rectangles upon which the curves appeared.

According to Bailey,[4] "the simplest method of determining the granulation of flour involves sifting a weighed quantity, say 100 grams, through a series of bolting silks nested one above the other in order of increasing coarseness."

Kress [5] performed the granulation test by sifting 100 g of flour for 8 minutes in a seven-sieve experimental sifter. The eight fractions that

[1] Received for publication May 9, 1938.

[2] CUTLER, G. H., and BRINSON, G. A. THE GRANULATION OF WHOLE WHEAT MEAL AND A METHOD OF EXPRESSING IT NUMERICALLY. Cereal Chem. 12: 120–129, illus. 1935.

[3] BALAKSHIN, S. THE FARINOGRANOMETER—A NEW DEVICE FOR GRAPHICALLY SHOWING THE FINENESS OF PRODUCT. Amer. Miller 64 (1): 14–15, illus. 1936.

[4] BAILEY, C. H. FLOUR. Paper given before Biscuit and Cracker Manfr. Assoc. p. 14. 1926. [Typewritten.]

[5] KRESS, C. B. GRANULATION OF FLOUR AND ITS RELATION TO QUALITY. Cereal Chem. 6: 202–214, illus. 1929.

remained on each sieve and pan were collected, weighed, and the percentage of each calculated.

Cutler and Brinson [6] were the first to use wheat meal instead of flour for measuring the degree of particle fineness or granulation of strains and varieties of wheat. The relative granulation of the meal was determined by sifting 50 g of wheat meal for 1 hour in a two-sieve Ro-Tap. The weight of the meal on each sieve, as well as that in the pan, was used in calculating the granulation number for each sample. Likewise, Brinson [7] reported correlation coefficients of +0.90 and +0.83 in the 1931 and 1932 samples, respectively, between the meal in the pan and the "granulation number."

Fifield et al.,[8] using the method suggested by Cutler and Brinson [6] with modifications in sieve size, grinding, and method of expressing the results, found wide differences in degree of meal fineness in wheat varieties grown in the western region.

MATERIALS AND METHODS

In the investigations undertaken to study technique, three varieties of wheat having a wide range in meal granulation were used. The varieties were, in the order of increasing particle fineness, Michikof, Michigan Amber, and Purdue No. 1. Two thousand grams of sound, clean, normal wheat of each variety were prepared and stored in tight galvanized boxes. Representative samples for the study of each step in technique were obtained by reducing the 2,000-g lot in a Boerner sampler to an appropriate size. All steps in the procedure were studied with both medium and fine wheat meal, ground with the Labconco mill set at F and XF, respectively.

Moisture content of the wheat varied from 9.6 to 10.1 percent. From 12 to 32 replications were made in appraising each variable involved in the procedure.

Except for the variable under investigation the following method was used: 50 g of wheat were ground with the Labconco mill and placed in an airtight glass bottle. From this lot representative 2.5-g samples were placed on the top sieve and sifted for 1 hour in the granulometer, illustrated and described in this paper. The material passing through the finer sieve or into the pan was weighed and expressed in percent of the total, and designated as particle-size index. The higher the index the finer the sample, and the lower the index the coarser the sample. All tests involving any one step in the technique were completed within 6 days.

In tests comparing the two methods commonly used in designating meal granulation, 197 F_2 hybrids originating from a Michikof × American Banner cross and selected at random, were used. These hybrids furnished samples that not only possessed a wide range in degree of meal fineness, but were also suitable for statistical analysis.

[6] CUTLER, G. H., and BRINSON, G. A. See footnote 2.

[7] BRINSON, G. A. THE RELATION OF GRANULATION OF WHOLE WHEAT MEAL TO "QUALITY" IN WHEAT WITH SPECIAL REFERENCE TO THE INFLUENCE OF FERTILIZER TREATMENT. 103 pp., illus. 1933. (Thesis submitted for M. S. degree, Purdue Univ.)

[8] FIFIELD, C. C., BODE, C. E., BAYLES, B. B., HAYES, J. F., WEAVER, R., and CHRISTIE, A. CHEMICAL MILLING AND BAKING RESULTS FOR WHEAT VARIETIES GROWN IN THE COOPERATIVE VARIETAL EXPERIMENTS IN THE WESTERN REGION IN 1934. U. S. Dept. Agr. 41 pp. 1936. [Mimeographed.]

——— BODE, C. E., BAYLES, B. B., HAYES, J. F., WEAVER, R., and CHRISTIE, A. QUALITY STUDIES OF WHEAT VARIETIES GROWN IN THE WESTERN REGION IN 1935. U. S. Dept. Agr. 24 pp. 1937. [Mimeographed.]

Coefficients of correlation and differences necessary for significance between two samples or means were calculated to aid in the interpretation of the data.

DESCRIPTION OF EQUIPMENT

In developing new equipment for meal-granulation determinations, particular emphasis was placed upon its suitability for testing a large number of samples and using small amounts of wheat meal as well as for assuring accurate and dependable results. Such equipment, if available, would greatly aid the plant breeder and geneticist in studying the relative meal fineness in wheat varieties and hybrids. A large

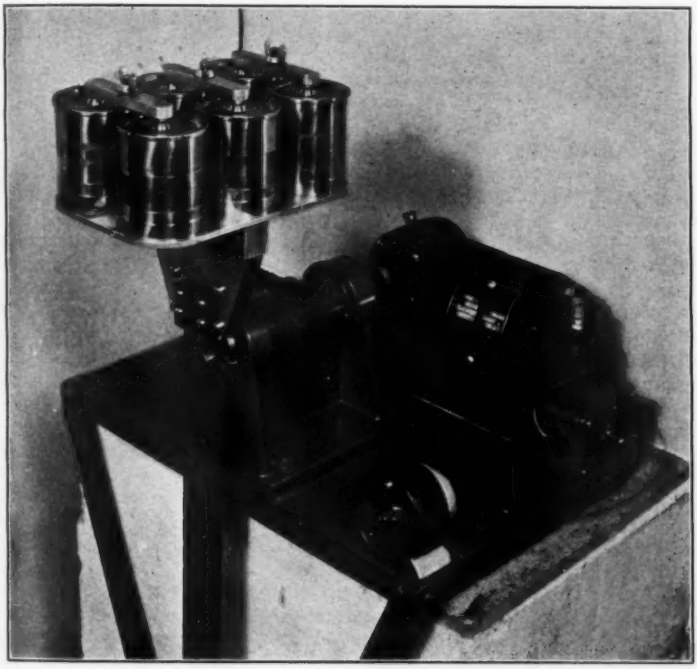


FIGURE 1.—Granulometer used for granulation tests.

number of preliminary experiments were conducted in which various types and sizes of motors, speeds of sifting, width of sieves, agitating devices, etc., were used. As a result of these tests, the granulometer shown in figure 1 was developed.

The granulometer is of simple, sturdy construction mounted on rubber floor cushions on a concrete block. Six nests of sieves are held firmly to the sieve carriage by bolts with wing nuts. Each nest of sieves includes the following: cover, two 2¼-inch sieve frames fitted with United States standard 60- and 270-mesh wire cloth, and a pan. Passage of the material through the sieves is accomplished entirely by the force of gravity. The sieve carriage rotates in a

vertical plane as a result of the eccentricity of the driving shaft, and is given a sudden jarring by a cam mounted on the outer end of the eccentric shaft. The impulses are generated by a ½₀-horsepower induction motor with built-in reduction gear which gives a speed to the main shaft of 216 revolutions per minute. A time switch is mounted on the base of the shaker. The granulometer is not only self-contained and cheap, but permits the testing of as many as 36 to 42 samples per day.

NATURE OF MEAL GRANULATION

It is a matter of common knowledge that flours derived from different classes and types of wheat differ in respect to granulation. Cutler and Brinson[9], using wheat meal, not only showed that granulation of the meal varied widely among different classes, but from a study of a large number of commercially grown soft and hard wheat varieties they demonstrated that bread flours originate from varieties that grind into a coarse meal, while pastry flours come from varieties that produce a fine meal.

Although considerable work has been reported on meal granulation, no information was found that showed the exact shape or form and nature of meal particles. In order to study the nature of the particles found in wheat meal, the meal fractions collected on top of the 60- and 270-mesh sieves and in the pan have been examined under the microscope. Studies were made with meal fractions representing varieties in the hard and soft wheat classes and with samples ground to varying degrees of fineness. In figure 2 are illustrated meal particles varying in size, shape, and angularity, of the three fractions originating from hard and soft wheats ground on the Labconco mill set at XF.

An examination of the individual fractions shown in figure 2 reveals a distinct difference in shape and angularity of the granules in the two samples. The granules in the hard wheat sample (*A*, *B*, and *C*) are characterized by sharp angular edges, while those in the soft wheat sample (*D*, *E*, and *F*) possess dull edges with rounded corners. The hard wheat sample is made up of gritty, vitreous particles, while those in the soft wheat are soft, starchy, and mealy in appearance. In addition to the great variations manifested in the meal granules, the proportion of meal collected on each sieve and pan varied greatly in the two samples. In the hard wheat, Michikof, 65 percent of the meal remained on top of the 60-mesh sieve, 25 percent on the 270-mesh sieve, and only 10 percent passed through the finer sieve and into the pan, whereas, in the soft wheat, Purdue No. 1, these proportions were 52, 28, and 20 percent, respectively. Moreover, flour milled from Michikof is of the coarse granular character usually associated with hard wheat flour, while that from Purdue No. 1 is soft, velvety, and smooth, characteristic of soft wheat flour.

EXPERIMENTS IN TECHNIQUE

The experiments dealing with technique were conducted over a period of 2 years, during which time more than 2,000 samples were sifted. The following steps in procedure were studied: (1) Numerical expression of meal granulation, (2) variation between tests, (3) varia-

[9] CUTLER, G. H., and BRINSON, G. A. See footnote 2.

tion among sieve sets, (4) duration of sifting, (5) size of sample, (6) amount of wheat ground, (7) speed of sifting, (8) moisture content of wheat, (9) age of meal, (10) fineness of grinding, and (11) experimental error in the test.

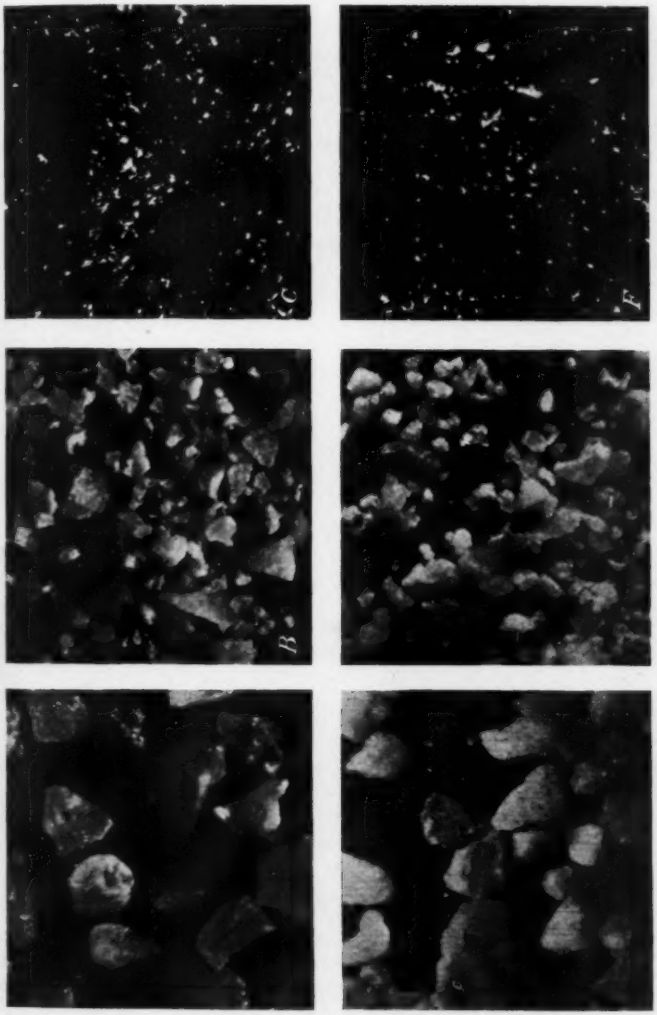


FIGURE 2.—Photomicrographs of coarse (*A* and *D*), medium (*B* and *E*), and fine (*C* and *F*) fractions of wheat meal. Fractions *A*, *B*, and *C*, representing a hard wheat, and *D*, *E*, and *F*, a soft wheat, illustrate the relative size, shape, angularity, and nature of particles or granules. × 20.

A large number of preliminary tests were conducted in which the various steps in procedure were tested in order to obtain information for carrying out the further tests which formed the basis for establishing a standard technique for measuring wheat meal granulation.

131451—39——2

Only the data from these later experiments are reported. In presenting the data, averages of the three varieties used are reported, except in cases in which the individual varieties did not react uniformly to the particular variable under investigation.

NUMERICAL EXPRESSION OF MEAL GRANULATION

Obviously, there are many advantages in numerically designating the fineness of a given wheat meal. Two methods of expressing granulation results have been reported; namely, (1) granulation number in which all fractions are used in its calculation,[10] and (2) percentage of material that passes through the finest sieve or into the pan.[11] In these studies a comparison was made of the two methods. Samples of meal from 197 F_2 hybrids of Michikof×American Banner, selected at random were used. The samples varied greatly in degree of fineness. The relation between granulation number and percentage of material in the pan, as well as the interrelation between the proportions collected on the 60-mesh sieve, 270-mesh sieve, and pan are shown in the following tabulation:

Correlations between—	*Coefficient of correlation and standard error*
Meal in pan and granulation number	+0.95±0.007
Meal in pan and meal on 270-mesh sieve	+.51± .053
Meal in pan and meal on 60-mesh sieve	−.97± .004
Meal on 270-mesh and on 60-mesh sieves	−.82± .024

The very high positive coefficient of correlation obtained between the percentage of material in the pan and granulation number indicates that the two methods are equally useful and efficient in numerically designating the relative fineness of wheat meal. However, inasmuch as fewer weighings are required and the subsequent calculations are greatly simplified, it was decided to use the percentage of material passing through the finer sieve or into the pan as an index of meal granulation. The percentage of the finest fraction was designated "particle-size index," and is used throughout this paper.

In comparing the relationship of the various proportions of coarse (on 60-mesh sieve), medium (on 270-mesh sieve), and very fine (pan) particle fractions resulting from the sifting tests of 197 hybrid samples, coefficients of correlation were calculated. The data show the following: (1) Increases in the proportion of very fine separations associated with increases in amount of medium-size fractions, or vice versa, (2) negative relation between the amounts of very fine and coarse meal fractions, and (3) negative relation between the medium and coarse particle fractions.

VARIATION BETWEEN TESTS

In determining the amount of variation that exists between different sieving tests, 16 separate tests were conducted in which medium and finely ground wheat meal were used. Each test included duplicate 2.5-g samples of Michikof, Michigan Amber, and Purdue No. 1, sifted for 60 minutes. The data obtained from each test were averaged and are shown in table 1.

[10] BRINSON, G. A. See footnote 7.
CUTLER, G. H., and BRINSON, G. A. See footnote 2.
[11] BRINSON, G. A. See footnote 7.
FIFIELD, C. C., BODE, C. E., BAYLES, B. B., HAYES, J. F., WEAVER, R., and CHRISTIE, A. See footnote 8, both references.

The results from the different sieving tests are very consistent. An experimental error of less than 1 percent was obtained with either medium or finely ground meal. The variability of this factor is smaller than that usually found in laboratory and chemical tests, and should have little effect on the end results.

TABLE 1.—*Variation between different sieving tests* [1] *with medium- and finely-ground meal from three varieties of wheat*

Test No.	Particle-size index of meal		Test No.	Particle-size index of meal	
	Ground medium	Ground fine		Ground medium	Ground fine
	Percent	*Percent*		*Percent*	*Percent*
1	10.9	18.3	6	10.8	18.4
2	10.9	18.7	7	11.0	18.5
3	10.8	18.6	8	10.7	18.7
4	11.0	18.6	Standard deviation	.11	.15
5	10.9	18.7	Coefficient of variability	.98	.82

[1] 12 samples used in each test.

VARIATION AMONG SIEVE SETS

Since six different sets or nests of sieves are employed on the carriage of the granulometer in determining the particle-size index of the meal, a study was made to determine the variation among the sieve sets and their location on the carriage. In studying this variable, 18 separate tests were made with medium and finely ground meal from three varieties of wheat. The data obtained are shown in table 2.

Small differences were found in the results obtained from the six different sets of sieves used in determining meal granulation. With medium ground meal the coefficient of variability was 0.92, while among sets in the fine meal it was 1.42 percent. No consistent relation was obtained between sieve sets and particle-size index either in the individual determinations or in the average of several tests.

DURATION OF SIFTING

To determine the duration of time of sifting needed for complete or nearly complete separation of the finest fraction, 2.5-g samples of meal were sifted for 15, 30, 45, 50, 60, 75, and 90 minutes in the granulometer.

TABLE 2.—*Variation in sieving tests* [1] *made with six different sets of sieves when medium and finely ground wheat meals were sifted simultaneously in the granulometer*

Sieve set No.	Particle-size index of meal		Sieve set No.	Particle-size index of meal	
	Ground medium	Ground fine		Ground medium	Ground fine
	Percent	*Percent*		*Percent*	*Percent*
1	11.0	17.6	5	10.8	17.2
2	10.9	17.6	6	11.0	17.0
3	10.8	17.2	Standard deviation	.10	.24
4	10.8	17.2	Coefficient of variability	.92	1.42

[1] 18 samples used in each test.

The average data obtained for the three varieties are shown in table 3.

TABLE 3.—*Influence of duration of sieving process upon the percentage of material sifted through a 270-mesh sieve from medium and finely ground wheat meal* [1]

Minutes sifted	Particle-size index of meal [2]		Minutes sifted	Particle-size index of meal [2]	
	Ground medium	Ground fine		Ground medium	Ground fine
	Percent	*Percent*		*Percent*	*Percent*
15	7.9	11.8	60	10.9	18.9
30	9.3	16.7	75	10.9	19.2
45	10.1	17.9	90	11.2	18.9
50	10.3	18.2			

[1] 12 samples used in each test.
[2] Difference necessary for significance between two means, odds 19:1=0.37.

The data in table 3 show a gradual increase in the percentage of material passing through the finer sieve as the time of sifting was increased from 15 to 60 minutes. No significant differences were obtained between samples sifted for 60, 75, and 90 minutes. Consequently, in all comparative granulation tests, samples of wheat meal were sifted for 60 minutes in the granulometer.

SIZE OF SAMPLE

In the development of a suitable technique for measuring meal fineness special attention was given to the possibility of testing small samples. A series of experiments was conducted in which 1-, 2-, 2.5-, 3-, 4-, 5- and 10-g samples were sifted for 60 minutes in the granulometer. The average results obtained from medium and fine meal of three varieties are reported in table 4.

The data show no significant difference between the results obtained with 1-, 2-, 2.5-, and 3-g samples. In the larger samples, especially the 10-g sample, complete separation of the finest fraction was not accomplished during the 60-minute period of sifting. In all regular tests, the 2.5-g sample has been adopted as standard, because of the ease in sampling, reliability, and suitability for testing a few grams of wheat.

TABLE 4.—*Influence of size of sample* [1] *of medium and fine meal on completeness of separation of finest meal fraction*

Meal (grams)	Particle-size index of meal [2]		Meal (grams)	Particle-size index of meal [2]	
	Ground coarse	Ground fine		Ground coarse	Ground fine
	Percent	*Percent*		*Percent*	*Percent*
1	10.7	17.4	4	9.9	17.6
2	10.7	17.5	5	10.0	17.7
2.5	10.6	17.6	10	10.0	17.3
3	10.5	17.8			

[1] 12 samples used in each test.
[2] Difference necessary for significance, 0.37.

AMOUNT OF WHEAT GROUND

From preliminary trials it was found that the quantity of wheat ground influenced the degree of fineness of the resulting wheat meal. To determine the effect of the amount of wheat ground on the percentage of the finest fraction or particle-size index, tests were made in which 5-, 10-, 15-, 25-, and 50-g lots of wheat were used. Separate lots of each sample size were ground with the Labconco mill set at F and XF to represent the medium and fine degrees of granulation. Since the varieties used did not react uniformly to the different amounts ground, the data for the three varieties are reported separately (table 5).

It will be noted that the size of the wheat sample ground greatly influenced the degree of particle fineness in the three varieties studied. In the soft wheat varieties Michigan Amber and Purdue No. 1, the larger the size of sample ground the finer was the meal, and the smaller the sample, the coarser the meal. With the hard wheat variety Michikof, on the other hand, the larger the quantity ground the coarser was the meal. Since the amount of wheat ground with the Labconco mill greatly affects the fineness of meal, it is necessary to grind samples of uniform size in all comparative tests. A 10-g sample is recommended. It may be that the 10-g sample will not prove best with all wheats and under all conditions. For the conditions of the present experiments, however, it serves as a basis for standardizing a suitable technique.

TABLE 5.—*Influence of size of sample* [1] *ground on the fineness of medium and fine meal from three varieties of wheat*

Wheat ground (grams)	Particle-size index of meal [2]					
	Ground medium			Ground fine		
	Michikof	Michigan Amber	Purdue No. 1	Michikof	Michigan Amber	Purdue No. 1
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
5	7.0	10.8	12.6	13.4	17.6	22.2
10	7.2	10.6	13.8	12.4	18.8	23.2
15	7.0	11.2	13.4	12.2	18.4	23.4
25	6.8	11.4	14.2	12.0	18.4	24.0
50	6.8	12.4	14.4	11.4	19.2	24.6

[1] 12 samples used in each test.
[2] Difference necessary for significance between 2 means, odds 19:1=0.68.

SPEED OF SIFTING

It was found, from preliminary trials, that a speed of at least 200 r. p. m. was necessary before a satisfactory sifting of meal was obtained. In later tests the speed of the shaft was 190, 200, 220, and 240 r. p. m. The data obtained are given in table 6.

The data show that, under the conditions of these experiments, complete or nearly complete separation of the finest meal fraction was accomplished at a rate of speed of 220 and 240 r. p. m. As a result of these tests, a speed of 216 [12] r. p. m. was selected in making the new equipment and was adopted as standard.

[12] Standard equipment.

MOISTURE CONTENT OF THE WHEAT

In determining the effect of moisture content of wheat on granulation of the meal, wheat samples varying widely in moisture content were used. Fifty-gram samples of each variety for each condition were placed in a humidifier and a dehydrator respectively for various periods of time. Immediately after the samples were taken out and ground, moisture-content determinations and sieving tests on the resulting meal were made. The data are shown in table 7.

TABLE 6.—*Influence of speed of sifting upon the completeness of separation of the finest fractions from medium and finely ground wheat* [1] [2] *meal*

Revolutions per minute	Particle-size index of meal [3]		Revolutions per minute	Particle-size index of meal [3]	
	Ground medium	Ground fine		Ground medium	Ground fine
	Percent	*Percent*		*Percent*	*Percent*
190	7.9	11.0	220	8.4	14.7
200	8.4	12.7	240	8.5	15.1

[1] Data are not comparable with those in other tables since the moisture content, source of samples, and the equipment used were different.
[2] 12 samples used in each test.
[3] Difference necessary for significance, 0.37.

TABLE 7.—*Influence of moisture content of the wheat used for grinding upon the percentage of material collected in the pan after sifting medium and finely ground meal* [1]

Moisture in wheat (percent)	Particle-size index of meal [2]		Moisture in wheat (percent)	Particle-size index of meal [2]	
	Ground medium	Ground fine		Ground medium	Ground fine
	Percent	*Percent*		*Percent*	*Percent*
7.6	9.1	16.2	10.0	10.4	17.6
8.7	10.5	17.3	10.9	12.0	20.8
9.0	10.3	17.4	12.4	11.7	[3] 16.7
9.6	10.3	18.1	13.3	12.3	[3] 15.3

[1] 12 samples used in each test.
[2] Difference necessary for significance, 0.37.
[3] A layer of meal formed on part of the 270-mesh sieve preventing complete separation of the fine meal.

Under the conditions of these experiments, wheat samples possessing a lower moisture content ground into a coarser meal than those of higher moisture content. The meal from samples containing 12.4 and 13.3 percent moisture formed a solid layer on a portion of the 270-mesh sieve. This prevented complete separation of the finest fraction and resulted in a lower particle-size index. Since the moisture content of wheat greatly influences meal granulation, it is necessary, in all comparative tests, to use samples of uniform moisture content. Considering all factors, wheat samples containing from 9 to 10 percent moisture have been found most suitable for granulation studies.

AGE OF THE MEAL

To determine the effect of age of meal on the results from granulation tests, 50-g samples of each variety were ground to the desired fineness and stored in airtight bottles. At weekly intervals representative samples from each lot were subjected to the regular sieving test. The results with meal stored for 0, 7, 14, 21, and 28 days are reported in table 8. They show that, in general, as the age of the meal increased, the particle-size index decreased. No significant difference is shown between the results obtained from meal tested immediately after grinding and that stored for 7 days in a tight bottle.

TABLE 8.—*Influence of age of coarse and finely ground wheat meal upon the results obtained in successive sievings made at weekly intervals* [1]

Aging period after grinding (days)	Particle-size index of meal [2]		Aging period after grinding (days)	Particle-size index of meal [2]	
	Ground coarse	Ground fine		Ground coarse	Ground fine
	Percent	*Percent*		*Percent*	*Percent*
0	10.6	18.1	21	9.8	17.1
7	10.9	17.8	28	10.1	17.1
14	10.3	17.8			

[1] 12 samples used in each test.
[2] Difference necessary for significance, 0.37.

FINENESS OF GRINDING

One of the main objectives in establishing a suitable technique for granulation studies was to emphasize the difference between hard and soft wheat samples. From preliminary trials it was observed that greater differences in granulation appeared between hard and soft varieties in finely ground wheat meal than in meal ground coarse. In order to obtain specific data on this point, sieving tests were conducted with coarse, medium, medium fine, and fine meal ground with the Labconco mill set at 1, F, between F and XF, and XF, respectively. The data for the three varieties, together with the amount of difference that exists between Michikof and Purdue No. 1, are given in table 9.

Great differences are noted in the degree of meal fineness caused by grinding the wheat coarse, medium, medium fine, and fine. The data show that the greatest differences between a hard and a soft wheat were obtained from the samples that were ground the finest. Since the difference necessary for significance between two individual samples is not much greater in the fine meal than in the coarser samples, and since there are obvious advantages associated with a greater difference between hard and soft heats, wheat ground into a fine meal was adopted and used in all regular tests.

TABLE 9.—*Influence of fineness of grinding of wheat samples upon the difference between results in sieving tests with meals from hard and soft wheats* [1]

Variety	Particle-size index of meal			
	Ground coarse	Ground medium	Ground medium fine	Ground fine
	Percent	*Percent*	*Percent*	*Percent*
Michikof	5.0	5.7	7.0	9.9
Michigan Amber	8.4	11.1	13.8	19.6
Purdue No. 1	10.5	13.8	16.6	24.3
Difference between Michikof and Purdue No. 1	5.5	8.1	9.6	14.4
Difference necessary for significance between 2 samples—odds 19:1	.60	.56	.54	.81

[1] 32 samples used in each test.

EXPERIMENTAL ERROR IN THE TEST

To determine the error that exists in the technique used, and the amount of variation that could be expected from sifting several hundred samples, 50 separate tests with meals from three varieties of wheat, ground medium and fine, were conducted over a period of 1 month. Variations caused by sampling, weighing, date of grinding, tests, sieves, and moisture content are involved. The data for each variety as well as the difference necessary for significance between two samples, in medium and fine meal, are shown in table 10.

A variation of about 5 percent is shown in the technique recommended for granulation studies of wheat meal. Although the wheat samples for this study were stored in a tight galvanized container, the moisture content in the wheat gradually decreased from 9.9 to 9.6 percent during the period of this investigation. As a result, the first samples tested possessed a somewhat higher particle-size index than those tested at the end of the period. Moisture content, therefore, caused a great part of the variability obtained in the 300 samples tested. The data show that differences of 1.24 for medium and 2.00 for fine meal are necessary for significance between two samples in comparing results obtained under conditions somewhat similar to those in this experiment.

TABLE 10.—*Experimental error in technique adopted for meal-granulation studies in tests of three varieties of wheat ground medium and fine* [1]

Fineness of grinding	Variety	Mean	Generalized standard deviation	Coefficient of variability	Difference necessary for significance between 2 samples: odds 19:1
Medium	Michikof	6.0	0.53	4.91	1.24
	Michigan Amber	12.1			
	Purdue No. 1	14.4			
Fine	Michikof	10.4	.86	4.80	2.00
	Michigan Amber	19.1			
	Purdue No. 1	24.1			

[1] 50 samples used in each test.

SUMMARY

Experiments dealing with a critical study of technique for measuring meal granulation were conducted over a period of 2 years during which time more than 2,000 samples were sifted.

A new apparatus, the granulometer, was developed, and the nature of meal fractions are illustrated and described.

Steps in the study of procedure included (1) numerical expression of meal granulation; (2) variation between tests; (3) variation among sieve sets; (4) duration of sifting; (5) size of sample; (6) amount of wheat ground; (7) speed of sifting; (8) moisture content of the wheat; (9) age of the meal; (10) fineness of grinding; and (11) experimental error in the test.

From the results obtained in a systematic study of each step in the procedure, the following technique for measuring meal granulation has been adopted: 10 g. of clean, sound, normal wheat, containing about 10 percent of moisture, are ground into a fine meal with the Labconco mill set at XF. Immediately after grinding, a 2.5-g sample is weighed and transferred to the larger sieve (60-mesh and 270-mesh sieves used) and sifted for 1 hour in the granulometer. The material that passes through the finer sieve or into the pan is weighed. The weight of this fraction is expressed as percent of the total and is designated as particle-size index. A low index indicates a coarse meal and a higher index indicates a relatively finer meal.

CAROTENE BALANCE AND BLOOD-CAROTENE LEVELS IN HEIFERS AND LACTATING DAIRY COWS, AND THEIR RELATION TO THE PRODUCTION OF OFF-FLAVOR MILK[1]

By C. H. WHITNAH, *dairy chemist*, and W. J. PETERSON, *assistant nutrition chemist, Department of Chemistry;* and F. W. ATKESON, *dairy husbandman in charge*, and H. W. CAVE, *dairy husbandman, Department of Dairy Husbandry, Kansas Agricultural Experiment Station*

INTRODUCTION

In the course of an investigation of oxidized flavor in milk, it was discovered that four cows were excreting in their feces more carotene [2] than they were consuming. The flavor of the milk from each of these cows was markedly and quickly improved by adding a carotene concentrate [3] to the ration.

Since Atkeson and his coworkers (*1*),[4] Fraps and Treichler (*10*), and Moore (*18*) had already shown that only a small fraction of the total carotene ingested appears in the milk, either as carotene or vitamin A, it was not altogether unexpected that large quantities of carotene should be excreted in the feces.

In view of the great losses in the metabolism of carotene, it seemed desirable to examine the nature and quantity of these losses under variable conditions. The relationship of the excretion of carotene in the feces of cows to carotene intake and to the concentration of carotene in the blood serum was studied. The results are presented in this paper.

Except for carotene and vitamin A secreted in the milk, these studies were balance trials. In a previous study, Atkeson and his coworkers (*1*) showed that when cows were stall-fed green rye only 0.086 percent of a daily intake of 3.5 g carotene was deposited in the butter, as carotene, and only 0.154 percent as vitamin A. From these figures one might conclude that a carotene balance of the type here presented would not be appreciably affected by the small amounts of carotene secreted in the milk either as carotene or as vitamin A. Harris and Ray (*14*) found that animals without adequate reserves of vitamin C would use a suddenly increased supply much more efficiently than would animals with larger reserves. It was totally unexpected that cows showing evidence of carotene deficiency or even cows with moderate reserves would excrete in their feces carotene from body stores. The analogy to vitamin C, and consideration from this standpoint of certain experiments on children and rats might, however, have suggested this possibility.

[1] Received for publication June 8, 1938. Contribution No. 233, Department of Chemistry, and No. 123, Department of Dairy Husbandry. Some of the material in this paper was presented orally at the Rochester meeting of the American Chemical Society, September 1937.

[2] The word "carotene," as used in this paper, refers to the petroleum-soluble pigment obtained by the methods described for the determination of β-carotene (*11, 19*).[4] There is some evidence that other pigments may be included in such a classification.

[3] This concentrate was a special preparation made by Nutritional Research Associates, Inc., by absorbing on wheat bran and middlings, a vegetable-oil solution of carotene previously obtained by extraction with fat solvents.

[4] Italic numbers in parentheses, refer to Literature Cited, p. 353.

REVIEW OF LITERATURE

Relatively little consideration has been given to the ability of the bovine to absorb and retain carotene. Such studies have, however, been conducted on people. Rowntree (*21*) found that children on a variety of diets lost in the feces 2 to 12 percent of the carotene ingested. Infants fed a diet very low in vitamin A potency excreted for several days a larger amount of the vitamin than they ingested. Vitamin A was not distinguished from carotene in this study.

According to some workers the absorption of carotene from the intestine depends upon the simultaneous presence of a suitable fat or oil. Wilson, Das Gupta, and Ahmad (*23*) found that carotene fed to the human in the form of raw carrots or cooked spinach was about 90 percent absorbed when the diet contained fat, but only 50 percent when fat was omitted.

Booth et al. (*5*) found that carotene in butter was less potent for rats than an equivalent amount of vitamin A.

Basu (*3*) reported that carotene administered to rats in olive oil was almost completely absorbed, but in arachis oil approximately 90 percent could be recovered. With the same dosage of carotene, the rate of growth depended upon the oil used as a solvent, being greatest when linseed oil was used and least when arachis oil was used, while mustard, olive, sesame, and coconut oils occupied intermediate positions as solvents.

Coward (*6*) on the other hand maintains that there is no difference in the power of animals to utilize either vitamin A or carotene when supplied to them in cod-liver oil, plant tissues, butters, or as concentrates in various vegetable oils, and that the causes of the differences in effectiveness of carotene dissolved in different oils must be sought elsewhere.

De (*8*), working with rats under varying conditions of feeding, found only 45 to 65 percent of ingested carotene absorbed. De points out that differences in body weights of the experimental animals, or in the amounts of fat, yeast, and minerals in the diets did not cause significant variations in the absorption of carotene.

Moore (*17*) found that the concentration of pigment in the feces fat of rats was 12 times the concentration of the carotene in the ingested palm oil. A most consistent feature of these experiments was the persistence of carotene apparently unchanged within the alimentary tracts of the animals.

Dutcher et al. (*9*) fed carotene (without mineral oil) to rats at six levels, varying from 0.001 mg to 0.006 mg daily. Carotene excreted in the feces was not proportional to the intake. A 500-percent increase in intake resulted in only approximately 50-percent increase in carotene recovered from the feces; the relative losses were greater at lower intakes.

Wilson, Ahmad, and Majumdar (*22*) fed 0.05 mg of carotene per day to rats previously depleted of vitamin A reserves. The average recovery in feces was over 50 percent when the carotene was given as a suspension in glucose solution, or with 5 percent of bile, or with 15 percent of meat.

To determine the efficiency of absorption of carotene, Davies and Moore (*7*) examined the feces and urine of rats which had been receiving approximately 8.2 mg of carotene in coconut oil daily for 34 days. The amounts found represented 5 to 10 percent of the ingested caro-

tene. This was less than one-tenth the relative loss observed by Wilson et al., but since the intake was 160 times that of Wilson's animals, the smaller relative loss was still 16 times the total loss of the animals on the lower intake. In the six tests reported by Dutcher et al. and also in the two tests reported, respectively, by Wilson et al. and by Davies and Moore the higher levels of intake accompanied the larger total loss. A smaller relative loss, however, prevailed with each larger intake.

Guilbert and Hart (*12*) have shown that carotene, the principal pigment of beef fat, may be withdrawn from the adipose tissue during vitamin A privation without a coincident reduction of the fat reserve. They also concluded that carotene in the fat of cattle constitutes a significant part of the total vitamin A reserve. Moore (*18*) pointed out that, in the case of the cow on pasture, the daily output of vitamin A and carotene in the milk fat is very small in comparison with the amount of carotene available in the diet. Storage of vitamin A in the liver takes place, while a large excess of pigment must undoubtedly be excreted unchanged. He also observed that the vitamin A content of the body fat remains within normal limits in the face of a large dietary excess of carotene.

Before presenting detailed results on the large amounts of carotene excreted, a few remarks regarding the reliability of the measurements may be appropriate. It is quite likely that 5 to 10 percent of the yellow pigment measured as carotene was xanthophyll which alcohol failed to remove (*16*). Such an error, however, should apply equally well to the analyses of the rations and the excreta. Even an unbalanced error of this magnitude would not change the significance of the results.

Of particular interest to this problem is the finding of pseudocarotenes or additional pigments soluble in petroleum. Quackenbush, Steenbock, and Peterson (*20*) found that under certain conditions xanthophylls may be converted into petroleum-soluble pigments. These workers isolated five new carotenoids, designated pigments A, B, C, E, and F, from alfalfa silage and acidified fresh alfalfa. Three of these, pigments A, B, and C, are epiphasic. The usual methods of carotene analysis failed to differentiate between carotene and pigments A, B, and C.

Wiseman, Kane, Shinn, and Cary (*24*) have reported irregularities in the absorption curves of such pigments and of the fractions separated chromatographically.

Kemmerer and Fraps[5] also found yellow pigments in the excrement of rats and chickens that had received feed containing practically no carotene. These coloring matters could not be separated from the carotene by the usual procedure for extracting carotene. The absorption curves of these pigments were very different from that of carotene even though they absorbed some light in the same region of the spectrum.

EXPERIMENTAL PROCEDURE

The animals used in the present study included 6 yearling heifers and 17 lactating cows. The breeds represented were as follows: Heifers—two Holsteins, three Jerseys and one Guernsey; cows—six Holsteins, three Ayrshires, four Jerseys and four Guernseys. The

[5] KEMMERER, A. R., and FRAPS, G. S. DIGESTIBILITY OF CAROTENE BY RATS AND CHICKENS. Presented before Div. Biol. Chem. at Dallas meeting of Amer. Chem. Soc. Abs. 29, B14. 1938.

various rations provided a range of carotene intake from 0.0028 to 2.51 mg per kilogram of body weight or a total daily intake varying from 1.25 to 1,252 mg. Some winter rations included silage made from Atlas sorgo as a carotene carrier.

Carotene supplements to the winter rations were provided either as dehydrated fresh green oats, or as the carotene concentrate previously mentioned. A summer ration which included green grass was changed during the test to an almost carotene-free ration of beet pulp and grain. The best and most strongly supplemented winter ration contained about two-thirds as much carotene as the ration which included some green grass or pasture. Unsupplemented good winter rations provided about one-eighth of this maximum. For several cows these rations were also supplemented to raise this ratio to about one-third. Poor winter rations supplying from one-fiftieth to one-tenth the maximum were fed for from 1.5 to 5 months. A minimum ration supplying only one-seven hundredth of the same maximum was fed for 2 weeks.

Spectrophotometric carotene determinations by the method of Guilbert (*11*) as modified at this station (*19*) were used throughout. Optical densities were determined at the proper wave lengths for the two maxima and the minimum of δ-carotene and had, in all cases, very nearly the correct ratio. The variations in amount of carotene excreted, both from day to day for a given individual, and from one individual to another on the same ration, were much larger than was expected (table 1). Differences between groups, however, were large enough to be significant in spite of these variations.

Separate parts of each ration fed were collected for carotene determinations. The daily consumption of each of these parts was weighed for each animal and the carotene intake calculated from these measurements. Feces samples were collected from each animal for 24-hour periods, weighed, mixed, and sampled for immediate analysis. Samples of blood from the jugular vein were collected daily and centrifuged, and the resulting serum was used for carotene analysis.

It is possible that certain ingredients of some rations contained compounds or adsorbates of carotene which were not extracted by the treatments used, although the best known methods of extraction were used. While some excess of carotene outgo over intake could be explained by assuming errors in extraction, the larger ratios could hardly be accounted for in this way. The low blood-carotene levels that accompany the rations measured as poor in carotene indicate that there was at least some significance in these measurements.

In all studies of the effect of change of ration, the first measurements were made well after the time considered necessary for a complete change of the contents of the alimentary tract (*2*, *15*). Several repeated tests at longer intervals after changing the ration indicated that the minimum intervals were sufficient. The two intervals of feeding beet pulp need not be interpreted as conflicting with this conclusion.

RESULTS

Daily variations that may be expected for a group of typical dairy cows are shown in table 1. The rations, the levels of blood carotene, the total average daily carotene intakes, the intake per kilogram of body weight, and the average daily excretions in feces for all trials are

recorded in table 2. Each value given in this table for output and for blood level is the average of determinations on 3 successive days.

The data in table 2 have been presented from the standpoint of the rations fed. The lowest level of carotene intake at which animals were maintained for sufficient time to establish a fairly constant rate of depletion (this excludes the depletion experiment 5, b and 5, c) was in trial 1 a. In this trial a group of heifers had been for 5 months on a ration of cottonseed meal and straw sprinkled with molasses. The average carotene ingested was 0.043 mg per kilogram of body weight, or a total of 12 mg daily. When silage was fed with this ration to another group of heifers (1, c), the average carotene intake was approximately five times as much as for the group that received no silage. The amount of carotene excreted, however, was only 3.2 times as much.

Also, when the carotene intake was suddenly and greatly increased the group on the lower level of carotene intake (1, b) responded more emphatically and efficiently than did the group which had received silage (1, d). The drop in the ratio of output over intake was much greater (210 to 82 percent) for the group without silage. The carotene balance became positive in the group without silage, whereas it was still negative in the group receiving silage, even though the latter had a slightly larger intake.

TABLE 1.—*Daily variations of feces weight, carotene output in feces, and blood carotene for three typical cows*

Cow No.	Weight	Day of test	Wet feces weight	Total fecal carotene	Blood [1] carotene per 100 ml of serum
	Kilogram		*Kilograms*	*Milligrams*	*Milligram*
483	386	First	18.8	78	0.140
		Second	21.8	81	.148
		Third	19.9	92	.128
		Average	20.2	84	.139
493	439	First	28.8	144	.536
		Second	19.2	108	.428
		Third	17.5	120	.420
		Average	21.8	124	.461
498	309	First	15.6	33	.080
		Second	17.8	29	.057
		Third	21.2	23	.032
		Average	18.2	28	.056

[1] From jugular vein.

Lactating cows (2, a), fed cottonseed meal, straw, and molasses for 6 weeks, showed a much better utilization of carotene, and the blood carotene values were approximately twice as large (0.075/0.041) as those for heifers (1, a) which had received the same rations for 5 months. This difference was possibly due to the residual effect of the cows having received good-quality alfalfa for 40 days preceding the shorter period of straw feeding. After adding carotene concentrate to the rations of both groups, 1, b and 2, b, the levels of carotene intake and the ratios of output over intake were very similar. The blood-carotene level for the lactating cows (2, b) now, however, was

TABLE 2.—*Variations in the carotene content of the blood and feces of dairy cows on different rations*

Series	Trial	Ration used	Period on ration	Milk flavor [1]	Cow		Daily carotene intake		Daily carotene output, total	Ratio output intake	Blood [3] carotene per 100 milliliter of serum
					No.[2]	Weight	Kilogram of body weight	Total			
						Kilogram	*Milligram*	*Milligram*	*Milligram*	*Percent*	*Milligram*
No. 1	a	Cottonseed, straw and molasses	5 months	(4)	144	405	0.037	15	22	147	0.027
					390	236	.047	11	27	245	.043
					391	257	.047	12	28	233	.039
					498	309	.042	13	28	215	.056
				Average		302	.043	12	26	210	.041
	b	Same as 1a, plus 225 g of carotene concentrate daily.	2 weeks	(4)	144		.212	86	85	99	.085
					390		.347	82	69	84	.097
					391		.368	95	63	67	.135
					498		.306	95	72	76	.175
				Average			.308	89	72	82	.122
	c	Cottonseed, straw, molasses, silage	5 months	(4)	147	400	.200	80	90	112	.175
					392	349	.254	56	73	130	.098
				Average		374	.227	68	82	121	.136
	d	Same as 1c, plus 225 g of carotene concentrate daily.	2 weeks	(4)	147		.382	153	155	101	.281
					392		.340	119	138	116	.151
				Average			.361	136	146	108	.216
No. 2	a	Cottonseed, straw, molasses	6 weeks, preceded by 40 days on alfalfa, corn, and bran.	Oxidized (except 109).	109	560	.064	36	61	169	.080
					249	471	.060	27	41	152	.080
					373	400	.068	27	25	93	.068
					464	423	.044	19	25	132	.074
				Average		463	.059	27	38	137	.075
	b	Same as 2a, plus 450 g of carotene concentrate daily.	2 weeks	Good	109		.322	181	157	86	.320
					249		.366	172	167	97	.317
					373		.432	173	148	86	.302
					464		.388	164	141	86	.298
				Average			.377	172	153	88	.309
No. 3	a	Yellow corn, bran, alfalfa	6 weeks, preceded by ration 2a for 40 days.	do	126	571	.218	125	137	110	.263
					240	510	.147	75	110	147	.201
					378	378	.195	74	84	113	.156
					483	386	.231	89	84	94	.139
				Average		461	.198	91	104	117	.189

No. 4	a	Yellow corn, oats, bran, linseed meal, silage, alfalfa.	5 months	do	112	624	.364	227	215	95	.317
					374	375	.294	110	135	123	.418
					493	439	.259	14	124	109	.461
				Average		479	.306	150	158	109	.398
	b	do	5 months	Oxidized	130	640	.211	135	214	158	.477
					134	456	.298	136	126	93	.488
					242	498	.278	139	150	108	.445
					370	435	.279	122	123	101	.400
				Average		507	.266	133	153	115	.452
				Average of 7.		495	.283	140	155	112	.429
	c	Same as 4a, plus 0.9 kg of dehydrated oats daily.	2 weeks	Good	150		.576	368	299	81	.658
					242		.744	371	261	70	.657
					370		.814	355	261	74	.401
				Average			.711	365	273	75	.572
	d	do	4 weeks	do	130		.578	369	369	100	.566
					242		.743	371	353	95	.585
					370		.511	354	304	86	.396
				Average			.711	364	342	93	.515
	e	Same as 4a, plus 1.8 kg of carotene concentrate daily.	2 weeks	do	134		1.53	697	544	78	.509
No. 5	a	Grain, alfalfa, silage, as in 3a, plus pasturage.	3 months	(5)	190	600	2.073	1,252	1,100	88	1.040
					486	450	2.511	1,130	980	87	1.420
				Average		525	2.292	1,191	1,040	87	1.230
	b	Linseed meal, molasses, beet pulp	1 week	do	190		.0031	1.88	28.1	1,495	.590
					486		.0028	1.25	18.1	1,450	.840
				Average			.0029	1.56	23.1	1,470	.715
	c	do	2 weeks	do	190		.0031	1.88	3.20	170	.246
					486		.0028	1.25	2.25	180	.265
				Average			.0029	1.56	2.72	175	.255

[1] The milk flavors recorded here were part of a study by Beck, Whitnah, and Martin (4).
[2] The breeds are indicated by the herd numbers used. Holstein=100+; Ayrshire=200+; Jersey=300+; Guernsey=400+
[3] From jugular vein.
[4] Heifers not in milk.
[5] Not tested.

131451—39——3

more than twice (0.309/0.122) that of the heifers (1, b). These results show that feeding histories for several months before tests are important in studies involving carotene metabolism, and that the maximum value may be attributed to the results only when the possible effects of factors other than current carotene intake have been considered.

When alfalfa with corn and bran was substituted for straw with cottonseed meal and molasses during a 6-week period (3, a) the carotene intake per kilogram of body weight was three times that of the straw-fed cows (3, a/2, a=0.198/0.059). For the alfalfa-fed cows the ratio of carotene output over intake differed but little from the ratio for straw-fed cows. The blood-carotene level, however, was more than twice as high, and the milk flavor was much better.

When cows receiving grain, alfalfa, and silage had their ration supplemented by the addition of 0.9 kg of dehydrated green oats daily (4, c), thereby more than doubling the carotene intake, great improvement in the utilization of carotene resulted. In 2 weeks the ratio of output over intake dropped from 115 percent to 75 percent, and the blood-carotene level increased from 0.452 to 0.572 mg. When the supplement was continued for 2 weeks longer a decrease in the efficiency of utilization occurred. The carotene excreted increased from 75 to 93 percent of the intake. The average blood-carotene level, however, only fell from 0.572 to 0.515 mg. A cow (4, e) receiving 1.53 mg of carotene per kilogram of body weight, or (about five times the intake of the cows receiving grain, alfalfa, and silage, and twice the intake of cows receiving the oats supplement (4, c), did not show a higher blood-carotene level nor did she utilize carotene more efficiently.

Cows under pasture conditions (5, a), and hence receiving a very much higher level of carotene (2.292 mg) than any of the others, had a positive balance and a remarkably high blood-carotene value (1.23). Cows on good winter rations, even with the largest amounts of dry carotene supplements, (4, c), reached a maximum blood level at an average value of 0.572 mg per 100 ml. It appears likely that prolonged feeding even at the highest carotene level used on winter rations would not have increased the blood-carotene level to that which was obtained under pasture conditions. It is entirely possible that other factors present in fresh green feeds may be responsible for what appears to be a more complete assimilation of carotene from pasture.

Although shortly after cows (5, b) were transferred from a summer ration to a winter ration their blood carotene was higher than the maximum produced with dry supplements, the higher value was rapidly declining and was not a maximum.

When cows which had been on a very high carotene intake from grass (5, a) were put on a ration practically devoid of carotene, the total fecal output of carotene dropped 98 percent the first week and 88 percent of the remainder during the second week. This final value does not appear to be a minimum, but it is insignificant compared with any other observed value. The blood-carotene, however, dropped only 42 percent the first week and 64 percent of the remainder the second. Even after 2 weeks on the carotene-free ration, the blood carotene was as high as that of many cows that had been receiving alfalfa and grain for 6 weeks. It would appear that the blood-caro-

tene level maintains itself for some length of time after the animal has been placed on a carotene-free ration.

In agreement with findings from studies on rats, the total amounts of carotene excreted were lower at the lower levels of intake, but the ratios of output over intake were higher. All the eight ratios above 160 percent accompanied intakes of less than 0.150 mg per kilogram of body weight; and twelve of the fourteen ratios above 125 percent accompanied intakes of less than 0.210 mg per kilogram.

In most cases the classification of lactating cows from the standpoint of rations has also classified them from the standpoint of milk which would or would not develop oxidized flavor. The tests with intakes below 0.075 mg per kilogram (2, a) involved milk that developed oxidized flavor. All but one of the cows in this trial group and all the heifers with intakes below 0.075 mg per kilogram were excreting more than 130 percent of their carotene intake. In the 12 tests with intakes above 0.300 mg per kilogram (2, b; cow 112 of 4, a, 4, c; 4, d; and 4, e) all the cows produced good-flavored milk and the output-over-intake ratios were all 100 percent or less. For intake levels between 0.100 and 0.300 mg the relation to milk flavor, and the relation to output-over-intake ratio were irregular.

In two groups of cows receiving the typical winter ration (grain mixture, alfalfa, and silage) for 5 months, and having nearly the same carotene intake (0.306 and 0.266), the milk flavor was good in the case of one group (4, a) while the milk from the other group (4, b) developed oxidized flavor. The group producing milk that developed oxidized flavor had a slightly higher blood carotene value and slightly lower intake. While neither of these differences considered alone seems sufficient to explain the difference in milk flavor, the difference in ratio of blood carotene to intake level is large enough to suggest that at these levels of intake the maintenance of a high level for blood carotene competes with the production of good-flavored milk. These two groups (4, a and 4, b) had a much higher blood carotene level (0.398 and 0.452/0.122 and 0.216) than heifers which were at about the same carotene-intake level after 2 weeks feeding of a ration containing cottonseed meal, straw, and molasses (0.308 for 1, b and 0.361 for 1, d) supplemented with a carotene concentrate. This indicates either that the heifers had a lower capacity to metabolize carotene or that 2 weeks was not a long enough period to bring the blood carotene up to the level maintained by an equal intake on a different ration.

That the carotene-intake level was not the only factor determining the tendency of milk to develop oxidized flavor is also shown by the fact that the five lowest carotene intakes per kilogram of body weight among cows producing milk that kept a good flavor (109 of 2, a, and all cows of 3, a, average=0.171) were below the three highest intakes among those cows producing milk which developed oxidized flavor (4, b, except 130 average=0.285). The first group had lower blood carotene levels (average=0.168) than the second (average=0.444). For cows producing poor milk on either poor or good winter rations, a sudden substantial increase in carotene intake produced both a rise in the level of blood carotene and an improvement in milk flavor. Possibly the oxidized milk flavor represents a tendency of some cows to care for the needs of their own bodies before protecting their milk. On the other hand it may be that the milk developed oxidized flavor, and the blood carotene level was high because these cows were not

efficient in transforming carotene into vitamin A. The former theory would more nearly fit the good flavor of milk from cow 109.

DISCUSSION

The daily carotene requirement of the bovine and other species according to Guilbert and Hart (*13*) is quite constant at 0.026 to 0.033 mg per kilogram of body weight. No evidence has been found that the physiological differences associated with lactation seriously affect carotene metabolism. In every case in this study, except that of cows receiving beet pulp and grain, the daily carotene intake was in excess of the minimum requirement. There is evidence, however, that many of the animals had less than an optimum supply of carotene. The frequent negative carotene balances, the existence of blood carotene levels far below those of cows on pasture, and the production of off-flavored milk which was corrected by feeding carotene supplements are such evidence.

Guilbert and Hart (*13*) also reported that the daily withdrawal of carotene from body stores when cows were on a vitamin-A-free ration was about 9 to 11 mg. From Guilbert's data (*11*) on carotene stores in body fat we might expect the straw-fed animals to maintain their measured carotene excretion for only 1 or 2 weeks. During the 6 weeks to 5 months preceding the tests both the output and the intake of carotene had probably been decreasing. The decrease of intake would be due to the slow decomposition of carotene in the rations. This loss might be proportional to the time of storage. The expected decrease of output would be due to the decreasing reserves and should decrease more nearly in a geometric ratio to time. It is likely then, that the excretion had decreased faster than the intake. It has been found (*17*) for rats that the intestines contain carotene several weeks after a carotene supplement has been withdrawn from an otherwise vitamin-A-free ration. While the intestines of cattle sometimes appear yellow, their carotene content is unknown. It may be assumed either that only a small fraction of the actual carotene intake was measured, that the bovine has some large unknown depot for the storage of carotene, or that something measured as carotene was produced within the animal. It is indeed fortunate that the relative absorptions at different wave lengths and the small minimum observed excretion satisfy so well tests proposed later by Wiseman et al. (*24*), and by Kemmerer and Fraps [6] to prove that the pigment was actually carotene.

SUMMARY

Spectrophotometric carotene determinations were made on rations, blood, and feces of cows on various rations with and without carotene supplements. The petroleum-soluble pigment obtained by the methods described for the determination of β-carotene was calculated as carotene. At levels of intake varying from 0.003 to 2.51 mg per kilogram of body weight, a relatively large amount of carotene was excreted in the feces. The average percentage of ingested carotene found in the feces of different groups varied from 75 to 1,470. The concentration of carotene in blood serum was found to vary greatly with the carotene intake and with the individual cow. The 3-day

[6] KEMMERER, A. R., and FRAPS, G. S. See footnote 5.

averages for carotene in blood serum varied from 0.027 to 1.42 mg per 100 ml.

Two groups of heifers were studied. Both received cottonseed meal and straw sprinkled with molasses. One group received, in addition, sorgo silage. The silage group receive five times as much carotene as the others, but excreted only 3.2 times as much. Supplements produced a much larger response in the nonsilage groups.

Cows which had received cottonseed meal, straw, and molasses for 6 weeks were compared with two other groups; one, the nonsilage-fed heifers, the other, a group of cows which received corn, bran, and alfalfa for 6 weeks following 6 weeks on the straw ration. The cows receiving cottonseed meal, straw, and molasses, showed better utilization of carotene and higher blood carotene levels than the heifers, and lower blood carotene levels than the alfalfa-fed cows. The two groups of cows, however, lost about equal proportions of their carotene intakes. The straw-fed cows responded to a supplement of carotene concentrate much better than the heifers. These results show that feeding histories for several months may be important in tests involving carotene metabolism.

The supplementing of a good winter ration of grain, alfalfa, and silage with carotene concentrate, dehydrated green oats, or fresh pasture grass showed greatly increased absorption of carotene in all cases. The most efficient utilization was observed 2 weeks after the dry supplements were first fed, but by 4 weeks the efficiency had dropped below that on fresh grass. It is entirely possible that factors in fresh grass, other than its carotene content, may be responsible for the better assimilation of carotene from the fresh grass.

When cows were transferred from a ration supplemented with pasture to a ration nearly devoid of carotene the fecal output of carotene dropped 98 percent the first week and 88 percent of the remainder the second week. The remainder after 2 weeks is insignificant compared with any other carotene excretion observed.

Low carotene intakes were quite regularly associated with milk which developed oxidized flavor, while high carotene intakes prevented or remedied this defect. The relation of flavor to moderate intakes was irregular. There is some indication that, with moderate carotene intake, the maintenance of the proper level of blood carotene competes with the protection of good flavor in milk, The level of carotene intake was possibly not the only factor determining the tendency of milk to develop oxidized flavor.

The optimum level of carotene intake appears to be many times the minimum intake required to protect from recognized deficiencies. The finding that the ratio of carotene output to intake increases at lower intakes is in agreement with data which other workers have reported.

LITERATURE CITED

(1) Atkeson, F. W., Hughes, J. S., Kunerth, Bernice L., Peterson, W. J., and Kramer, Martha.
1937. Recovery of carotene and vitamin A from butter when cows were fed unlimited quanitities of green rye. Jour. Nutrition 14: 621–629.

(2) ——— Hulbert, H. W., and Warren, T. R.
1934. Effect of bovine digestion and of manure storage on the viability of weed seeds. Jour. Amer. Soc. Agron. 26: 390–397.

(3) Basu, N. K.
1937. vitamin a and fat metabolism. Ztschr. Vitaminforschung 6: 106–110, illus. [Conclusions also given in German and French.]
(4) Beck, G. H., Whitnah, C. H., and Martin, W. H.
1939. relation of vitamin c, lecithin and carotene content of milk to the development of oxidized flavor. Jour. Dairy Sci. 22: 17–29, illus.
(5) Booth, Roland Gordon, Kon, Stanislaw Kazimierz, and Gillam, Albert Edward.
1934. the relative biological efficiencies of the vitamin a and carotene of butter. Biochem. Jour. 28: [2169]–2174.
(6) Coward, Katharine Hope.
1936. the utilization by the rat of vitamin a and carotene administered in different media. Biochem. Jour. 30: 1878–1882.
(7) Davies, Alan William, and Moore, Thomas.
1934. vitamin a and carotene. xi. the distribution of vitamin a in the organs of the normal and hypervitaminotic rat. Biochem. Jour. 28: [288]–295.
(8) De, N. K.
1937. the assimilation of vitamin a and carotene by rats from some common foods with a note on the conversion factor i. u./e., proposed by the international vitamin conference. Indian Jour. Med. Research 24: 751–766.
(9) Dutcher, R. Adams, Harris, Philip L., Hartzler, Eva R., and Guerrant, N. B.
1934. vitamin studies. xix. the assimilation of carotene and vitamin a in the presence of mineral oil. Jour. Nutrition 8: 269–283, illus.
(10) Fraps, G. S., and Treichler, Ray.
1932. quantitative variations in vitamin a content of butter fat. Indus. and Engin. Chem. 24: 1079–1081.
(11) Guilbert, H. R.
1934. determination of carotene as a means of estimating the vitamin a value of forage. Indus. and Engin. Chem., Analyt. Ed. 6: 452–454.
(12) ——— and Hart, G. H.
1934. storage of vitamin a in cattle. Jour. Nutrition 8: 25–44, illus.
(13) ——— and Hart, G. H.
1935. minimum vitamin a requirements with particular reference to cattle. Jour. Nutrition 10: 409–427.
(14) Harris, Leslie J., and Ray, S. N.
1935. diagnosis of vitamin-c subnutrition by urine analysis, with a note on the antiscorbutic value of human milk. Lancet [London] 228: 71–77, illus.
(15) Maynard, L. A., McCay, C. M., and Madsen, L. L.
1936. the influence of food fat of varying degrees of unsaturation upon blood lipids and milk fat. Jour. Dairy Sci. 19: 49–53, illus.
(16) Miller, Elmer S.
1935. a rapid and accurate quantitative method for the determination of the common carotenoids; analysis of beta-carotene and leaf xanthophyll in thirteen plant tissues. Jour. Amer. Chem. Soc. 57: 347–349.
(17) Moore, Thomas.
1931. vitamin a and carotene. vii. the distribution of vitamin a and carotene in the body of the rat. Biochem. Jour. 25: [275]–286, illus.
(18) ———
1932. vitamin a and carotene. ix. notes on the conversion of carotene to vitamin a by the cow. Biochem. Jour. 26: 1–9, illus.
(19) Peterson, Walter J., Hughes, J. S., and Freeman, H. F.
1937. determination of carotene in forage. a modification of the guilbert method. Indus. and Engin. Chem., Analyt. Ed. 9: 71–72.

(20) QUACKENBUSH, F. W., STEENBOCK, H., and PETERSON, W. H.
1938. THE EFFECTS OF ACIDS UPON CAROTENOIDS. Amer. Soc. Biol. Chem. Proc. 32: xcviii–xcix. [Included in Jour. Biol. Chem. v. 123, No. 3.]
(21) ROWNTREE, JENNIE I.
1930. A STUDY OF THE ABSORPTION AND RETENTION OF VITAMIN A IN YOUNG CHILDREN. Jour. Nutrition 3: 265–287.
(22) WILSON, H. ELLIS C., AHMAD, BASHIR, and MAJUMDAR, BIRENDRA NATH.
1936. FURTHER OBSERVATIONS ON THE METABOLISM OF CAROTENE. Indian Jour. Med. Research 24: 399–409.
(23) ——— DAS GUPTA, S. M., and AHMAD, BASHIR.
1937. STUDIES ON THE ABSORPTION OF CAROTENE AND VITAMIN A IN THE HUMAN SUBJECT. Indian Jour. Med. Research 24: 807–811.
(24) WISEMAN, HERBERT G., KANE, EDWARD D., SHINN, LEO A., and CARY, C. A.
1938. THE CAROTENE CONTENT OF MARKET HAYS AND CORN SILAGE. Jour. Agr. Research 57: 635–669, illus.

THE ANATOMY OF SPRUCE NEEDLES[1]

By HERBERT F. MARCO[2]

Junior forester, Northeastern Forest Experiment Station,[3] Forest Service, United States Department of Agriculture

INTRODUCTION

In 1865 Thomas (*16*)[4] made a comparative study of the anatomy of conifer leaves and found that the structural variations exhibited by the different species warranted taxonomic consideration. Since that time leaf anatomy has become a fertile and interesting field of research. Nearly all genera of gymnosperms have received some attention, and the literature on this subject has become voluminous. A detailed review of the literature will not be attempted in this paper, since comprehensive reviews have already been published by Fulling (*6*) and Lacassagne (*11*). Fulling's paper contains in addition an extensive bibliography on conifer leaf anatomy.

Most workers in this field of research have confined their efforts to the study of the cross sections of needles. This is partly because longitudinal sections are difficult to obtain and partly because they present but little structural variation of value for identification. The workers who have studied both longitudinal and cross sections have restricted their descriptions of longitudinal sections either to specific tissues or to a few species of a large number of genera, and the descriptions, although comprehensive, leave much to be desired from the standpoint of detailed information and illustration.

Dorner (*3*) was perhaps the first to use sketches to augment keys to and descriptions of the native firs and spruces. His diagrammatic sketches portray the shape of the needles in cross section and the position of the resin canals. Durrell (*4*) went a step further and illustrated his notes on the North American conifers by camera-lucida drawings depicting the orientation and arrangement of the various needle tissues in cross section.

Since the development of technique for taking them, photomicrographs have largely supplanted drawings. Such photographs are valuable aids in identifying and recording species, as can readily be appreciated by reviewing the works of Harlow (*9*), Fulling (*6*), and Sutherland (*15*).

The pines (*Pinus* spp.) and the firs (*Abies* spp.) have been the subjects of most conifer leaf studies. A few authors, however, have directed their attention to the study of the foliar anatomy of the spruces (*Picea* spp.), among them Burnet (*2*), Bastin and Trimble (*1*), Hayata and Satake (*10*), Lacassagne (*11*), and Marco (*12*). The studies of these authors were based upon cross sections. In none of their papers appears a complete description of the internal structures of spruce leaves. Further, there is considerable disagreement among the writers

[1] Received for publication June 16, 1938.
[2] Acknowledgment is made to A. W. Evans, of Yale University, for many helpful suggestions during the course of this investigation.
[3] Maintained by the U. S. Department of Agriculture at New Haven, Conn., in cooperation with Yale University.
[4] Italic numbers in parentheses refer to Literature Cited, p. 367.

concerning the shape of the needles in cross section, the number and position of resin canals, and the relative merits of various diagnostic characters.

It is the purpose of the present paper to describe in detail the internal structures of spruce needles as seen in both cross and longitudinal sections and to explain something of their functions. Where possible these structures will be illustrated by photomicrographs.

THE ANATOMY OF SPRUCE NEEDLES

For convenience the internal structures of a spruce needle may be divided into three general tissues—the dermal, the mesophyll, and the vascular. The dermal tissue embraces the epidermis and hypodermis. The mesophyll comprises the green, usually isodiametric cells which form the bulk of the needle. The vascular tissue comprises the endodermis, the transfusion cells, and the fibrovascular bundles. The resin canals are located in the mesophyll adjacent to the dermal tissue (pl. 1, *A*).

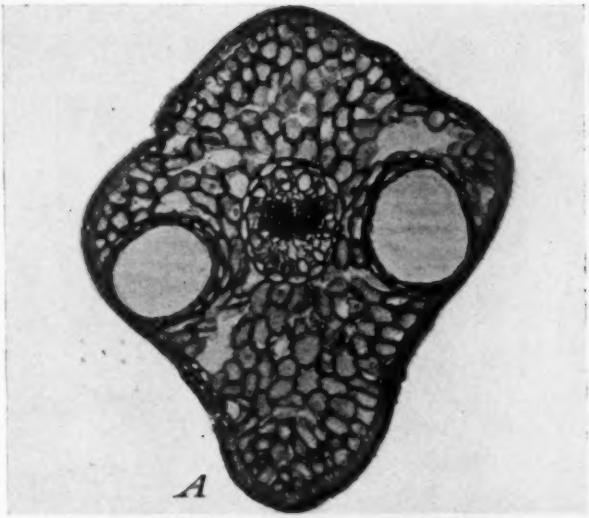
THE EPIDERMIS

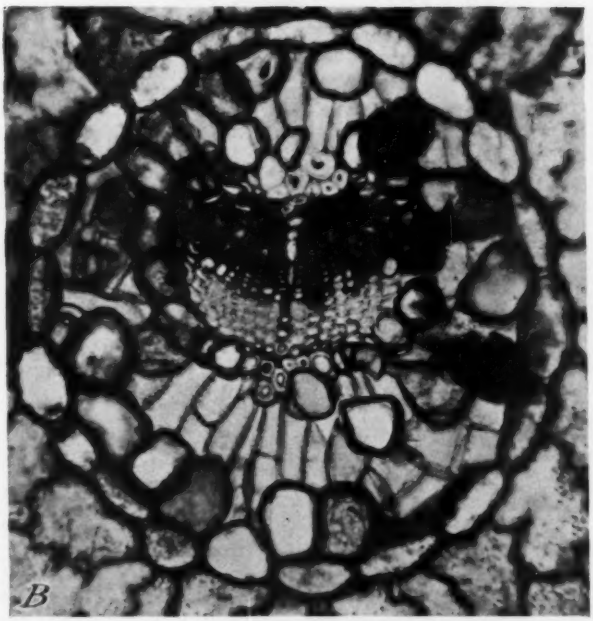
The epidermis is a single peripheral layer of cells, continuous except for stomatal openings. The cells, which are irregular in shape and size (pl. 2, *A*), are firmly fastened to one another by the interlocking of their walls. They measure approximately two to three times as long as they are wide; are half as thick as wide; and are oriented with their long axis parallel to that of the needle.

The lateral, cross, and inner walls of the cells appear equally and uniformly thickened. The outer walls, which arch outwardly, are in part considerably thicker than the other walls. The thickest portion of the outer walls is near the center of the arches. From this region the walls gradually become thinner toward their margins until they are of the same thickness as the lateral and cross walls.

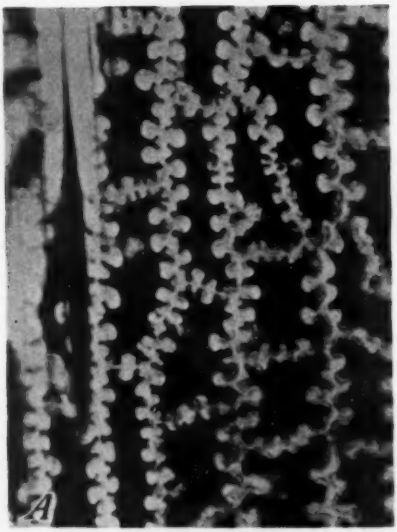
The epidermal cells lock together in an unusual manner. As seen in plane section, the lateral and cross walls appear incised or mortised at regularly spaced intervals (pl. 2, *B*). The outer portion of the cell wall between the mortises is slightly gabled. Extending outward from the peak of the gable is a narrow, tenonlike projection of the wall. These projections or tenons are slightly wedge-shaped, with the outer extremities wider than the inner. The walls of adjoining epidermal cells are constructed in the same manner, but the tenons of one cell are opposite the mortises of another. Consequently the epidermal cells are held together by a modified dovetailed joint. This explains why the epidermal cells cling tenaciously together even after a prolonged treatment in Jeffrey's or Schultze's macerating fluid. The walls of *Picea breweriana* S. Watson, in addition to being mortised and tenoned, are infolded.

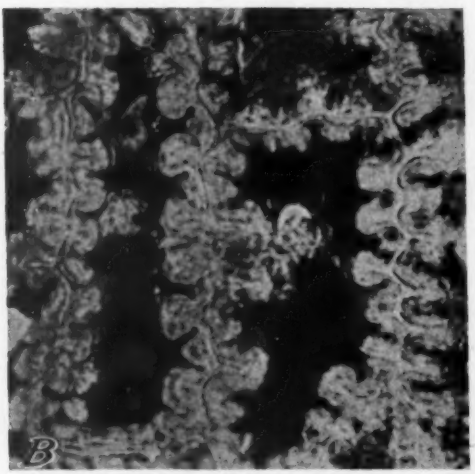
Around the periphery of the epidermal cells, immediately above and part of the cell wall between the mortises, are a number of toothlike projections which extend outwardly into the cuticle. The teeth, as seen in longitudinal sections, are variable in shape, ranging from square to mushroomlike. The latter type, which is the more common, also shows considerable diversification of crown forms. The crowns may be pyriform, hemispherical, or divided into rounded, hooked lobes. The teeth in *Picea breweriana* are accentuated by the infolding of the cell wall between the denticles (pl. 2, *C*).



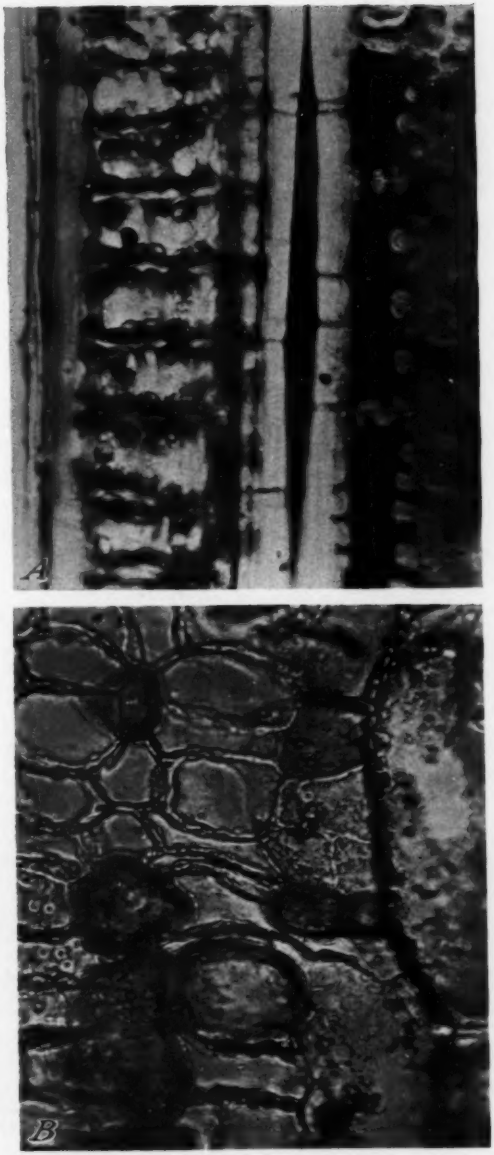


A, A typical cross section of a needle of *Picea engelmannii* (Parry) Engelm. Beginning at the circumference, the various tissues depicted are as follows: The outer peripheral row of cells is the epidermis; the second peripheral row of cells is the hypodermis; the interruptions in the epidermis and hypodermis are the stomata; within the hypodermis is the mesophyll; the ring of cells near the center of the needle is the endodermis; within the endodermis are the fibrovascular bundle and transfusion cells. Note the two large resin canals in the lateral angles of the needle. × 75. *B*, The central portion of a cross section of a *P. breweriana* needle showing the endodermis, transfusion cells, and fibrovascular bundle. The upper portion of the bundle is the phloem; the lower, the xylem. Note the strengthening cells or fibers above, below, and adjacent to the bundle. × 210.

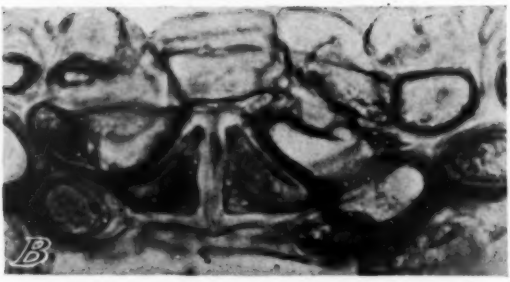


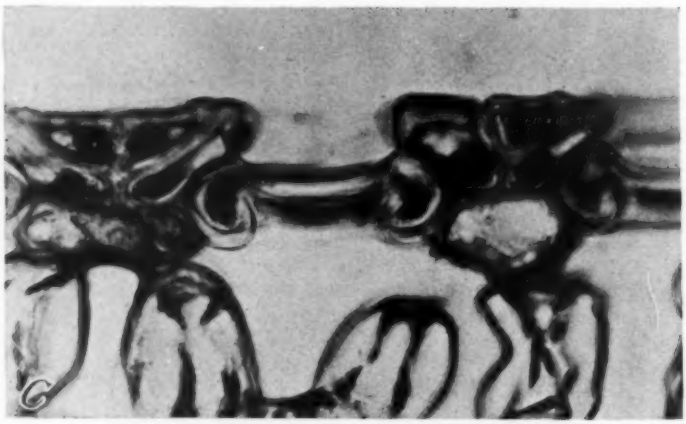


A, Tangential section through the epidermal cells of *Picea breweriana* showing the structure of the cell walls, × 350; *B*, a portion of the same section at a higher magnification to show the method by which the cells are locked together, × 890; *C*, longitudinal section through the epidermal cells of *P. rubra* Link, depicting the teeth on the outer walls, × 890.



A, Longitudinal section through the dermal region of *Picea breweriana* illustrating the simple pits and fusiform lumen of a hypodermal cell (center); the infolding of the epidermal cell walls (right); and the chloroplasts in the mesophyll cells (left). × 440. *B*, Longitudinal section through the transfusion tissue of *P. breweriana* portraying the border-pitted tracheal cells and the thick-walled, simply pitted endodermal cell (right). × 400.





A, Cross section through a stoma of *Picea rubra* depicting the excessively thickened upper walls of the accessory cells and the two elliptical guard cells. Note that the upper walls of the guard cells are distinct from the lower walls. The section was made near the center of the stoma. × 890. *B*, Cross section of the stoma shown in *A* near the ends of the guard cells. Note that the guard cells have become right-triangular in shape. × 890. *C*, Longitudinal section through a stoma of *P. rubra*.

In cross section these structures appear as a double row of rounded wedges inserted between two epidermal cells. They do not usually protrude into the cuticle beyond the arch of the outer cell wall except in some species, e. g., *Picea breweriana* and *P. asperata* Masters. The denticulations become almost indistinguishable in plane sections, since they merge with the cell walls in the background. When discernible the teeth are oblong in outline.

The cross, lateral, and tangential walls of the epidermal cells contain simple pits (pl. 2, *C*) by means of which the cells communicate with one another and with cells of the adjacent hypodermis. Blind pits that lead to the cuticle can occasionally be detected in the outer walls. Feustel (*5*) intimates that the epidermal pits are always closed, but no evidence was found in the present study to support this contention.

The epidermal cells contain large, conspicuous nuclei and thin cytoplasmic layers. The nuclei are generally found near the centers of the cells and are held in position by a number of minute cytoplasmic threads. The cytoplasmic layers are very thin and are difficult to distinguish from the cell walls, to which they closely adhere. The vacuoles, which fill most of the cell cavity, frequently contain tanniferous substances that often impart a brownish tinge to the cell contents and render them opaque.

Overlying the epidermis and continuous except for the stomata is the cuticle. This waxy layer, although universally present, varies in thickness with different species. For example, in *Picea breweriana* the cuticle is as thick as the epidermal cells, while in *P. spinulosa* (Griffith) Henry, it is much thinner and barely distinguishable from the cell walls upon which it lies. However, the cuticle is always sufficiently thick to cover the epidermal teeth. According to Gauba (*7*) the cuticle consists of two layers. The outer layer appears as a homogeneous, tough, waxy film; the inner is composed of small, densely packed, waxy particles. These particles enclose the teeth and closely follow the contours of the outer walls of the epidermal cells. The structure of the cuticle can be seen readily in *P. breweriana*.

THE HYPODERMIS

Beneath and in contact with the epidermis lies the hypodermis. This tissue, like the former, is continuous except where interrupted by the stomata and resin canals. The hypodermis, however, is composed of libriform fibers and frequently is more than one cell thick, especially in the angles of the needles. The fibers are often septate; are long to very long and thick to very thick walled; and have numerous simple pits and narrow lumina. In cross section the lumina of the fibers, which appear isodiametric, vary in size. The variation is caused by the shape of the lumina. As seen in longitudinal section, the lumina are narrowly spindle-shaped and occupy the middle third of the fibers (pl. 3, *A*). The remaining two-thirds of the fibers are composed almost entirely of cell-wall substance devoid of pits. Consequently, in a given cross section the lumina may vary from minute to large, since the fibers overlap.

The simple pits, of the flattened funnel-shaped type, radiate out from the lumina in all directions. Hence by means of the pits the fibers communicate with one another, with the epidermis, and with the mesophyll.

The cytoplasm of the fibers often stands out clearly and appears devoid of vacuoles. At times the cytoplasm becomes amber colored, owing to tanniferous infiltrations. The nuclei are large and are generally pressed against the cell walls.

THE STOMATA

Stomata occur along the lateral sides of all spruce needles with the exception of the species in the section *omorika*. In this section the needles are flattened, that is, compressed dorsiventrally, and the stomata are generally found only on the morphological upper side. An exception is *Picea sitchensis* (Bongard) Carrière, which though assigned to this section frequently has stomata on all sides of the needles.

The stomata are arranged in parallel rows extending in the direction of the needles. The number of rows on a given side of a needle is fairly constant in the same species but often varies considerably among different species. For example, in *Picea spinulosa*, 2 rows usually occur, while in *P. polita* Carrière up to 10 have been observed.

The stomata are typically xerophytic, that is, below the surface of the epidermis. In surface view the outer stomatal cavity is delimited by four specialized epidermal border cells. The two lateral border cells are kidney-shaped and extend in the direction of the needle. At either end and separating the lateral cells is a square or trapezoidal end border cell. The arrangement of these border cells imparts a broadly oval or barrel-shaped outline to the stomatal cavity. The outer walls of the border cells—those toward the cavity—appear to be excessively thickened, though actually they are uniformly thickened. The apparent greater thickness is caused by the upward thrust of the accessory cells, which will be discussed in the following paragraphs.

In thick plane sections and by proper downward focusing of the microscope two or four accessory cells may be seen. When two such cells are present, they extend from beneath the center of one end border cell to a similar position below the other, following the contours of the lateral border cells. When four accessory cells are present, they conform in shape to the border cells. The accessory cells are generally broader than the border cells.

Again, by downward focusing in these sections, two kidney-shaped guard cells may be discerned. They also extend in the direction of the needle and are oriented with their concave surfaces toward each other. They are approximately three-fourths as long as the accessory cells.

In cross section the lateral border cells and the accessory cells are roughly triangular in shape. The inclined or lower walls of the border cells slope downward and away from the outer stomatal chamber. The inclined walls of the accessory cells slope in the same direction, but in this case they represent the upper walls. Hence, the inclined walls of the border and accessory cells (in pl. 4, *A*, the cells on either side of the outer stomatal cavity) are joined.

The walls of the accessory cells are unequally thickened. The upper or inclined walls and the outer walls are greatly thickened, their thickest portion being in the region of their junction. From this region the walls gradually thin until they are almost as thin as

the lower membranous walls, which can be distinguished only with the aid of an oil-immersion lens.

The length of the inclined walls of the accessory cells is greater than that of the border cells. Hence the inclined walls of the accessory cells project beyond the border cells into the outer stomatal cavity, frequently to the outer surface of the epidermis. It is this projection of the inclined walls of the accessory cells that causes the outer walls of the border cells to appear thickened in plane section.

The structure of the guard cells in cross section is unique. To appreciate it fully, the guard cells should be observed when they are fully expanded and at high magnifications (pl. 4, *A*). In this condition the cells are somewhat teardrop-shaped and are oriented with their rounded or blunt ends toward the stoma. The cells are in contact with the accessory and adjacent cells only along the inner half of the upper wall (that toward the epidermis) and at the extreme inner end of the lower wall. The latter wall may be described as hinged to the hypodermis.

The upper and lower walls curve toward each other at both ends and are connected at the stomatal opening by means of a flexible membranous wall. The membranous wall can be best observed with the aid of an oil-immersion objective.

In 1898 Schwendener (*13*) observed a pitlike structure near the extreme inner end of the upper walls of the guard cells. This is actually a second membranous wall, as is shown by the fact that when the guard cells contract the membrane folds so that the upper wall comes in contact with the lower. So far as can be ascertained, pit membranes do not behave in this manner. When the guard cells are not expanded, the membranous walls adjacent to the stoma appear as dark lines separating the upper and lower walls. In this condition the guard cells assume an elliptical shape in cross section.

The cross-sectional shape of the guard cells near their ends, where they have not separated, is considerably different from that near the center of the cells. Near their ends the cells are right-triangular with the lower walls paralleling the epidermis; the upright or contact walls are at right angles to the lower walls, and the inclined walls slope downward and away from the outer stomatal cavity (pl. 4, *B*). The walls are uniformly thickened, and the membranous walls described previously are not in evidence. However, near the center of the two contact walls there is a thickening of the middle lamella, which evidently corresponds with the outer membranous walls.

THE RESIN CANALS

Two longitudinal series of resin canals occur in the leaves of all species of *Picea*. They are found in the mesophyll adjacent to the dermal tissue and in most species are immediately below the lateral angles of the needles. In the section *omorika* the canals are located on the lower side of the needles at various positions between the lateral and lower angles. The position of the canals is constant in the different species and often serves as a diagnostic character.

The resin canals rarely extend the full length of the needles. They usually occur as longitudinal, fusiform cysts separated by mesophyll tissue. The canals range from 1 to several millimeters long, averaging approximately 2 mm. In some instances a canal on one side of the needle may be continuous for several millimeters, while the canal

on the opposite side is broken into two or three widely separated short cysts.

Thus, in a given cross section, no, one, or two resin canals may be present. This feature, which will be discussed in a later paragraph, is important from the standpoint of identification.

Except in two species, the resin canals, as seen in cross section, normally always border upon the epidermis (pl. 1, *A*). The two exceptions are *Picea polita* and *P. sitchensis*. The resin canals in these two species touch upon the hypodermis. The cross sections show further that the diameters of resin canals vary considerably with different species. For example, in *P. sitchensis* the canals measured up to 440 microns, while in *P. likiangensis* Pritzel the largest was only 70 microns.

The resin canals are delimited by two generally uniseriate sheaths of cells. The outer or strengthening sheath is composed of short, thick-walled, fiberlike cells. The cells in cross section are flatly oval and are laterally in contact with one another. The cells are smaller than those of the hypodermis and their walls frequently display a laminated structure.

The strengthening cells are approximately one-third to one-half as long as the hypodermal fibers and have tapering or sharply inclined end walls. The cells with tapering ends overlap. This overlapping causes the sheath to appear biseriate in cross section. The cells with sharply inclined end walls are oriented in vertical series and their end walls abut squarely upon each other. Funnel-shaped simple pits with vertical, elliptical, inner apertures radiate from the lumina in all directions.

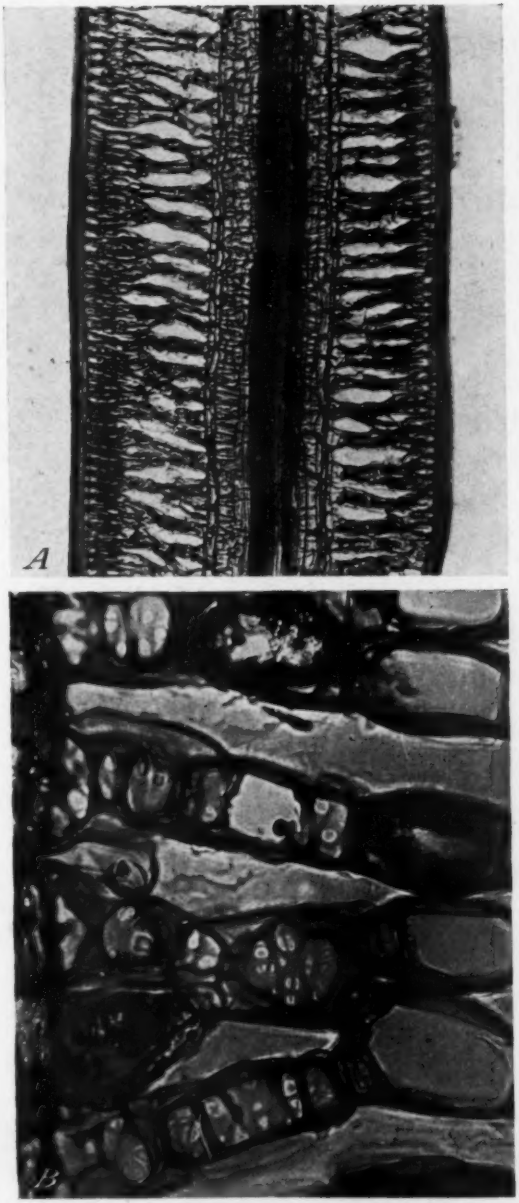
The inner or epithelial sheath is composed of short, thin-walled parenchymatous cells, which are approximately one-half as long as the strengthening cells and extend in the direction of the canals. The cells are somewhat hourglass-shaped and have sloping end walls. The end, lateral, and outer walls are thicker than the inner walls, which often bulge into the canals forming tylosoids. The walls contain numerous round, simple pits which often appear bordered. The bordered appearance of the pits occurs when the inner aperture is larger than the outer. Pits are not as numerous in the inner walls as in the other walls. In cross section the cells are thinly rectangular and wider than the strengthening cells to which they closely adhere.

The cells contain abundant whitish, translucent protoplasm with lenticular nuclei. According to Hannig (*8*) no resinous contents could be found in these cells. He reports that Schwaback, however, using copper acetate, detected fine particles of resin abundant everywhere in the epithelial cells.

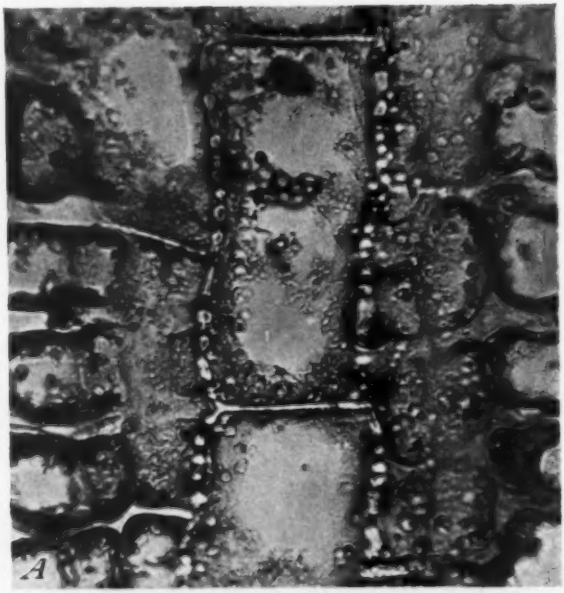
THE MESOPHYLL

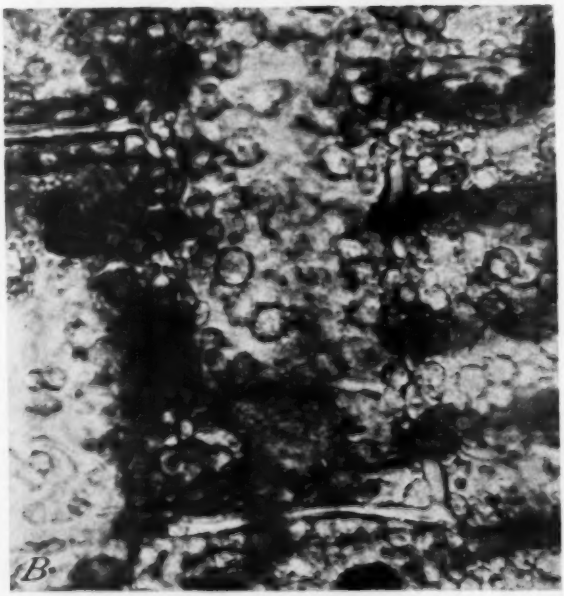
Within the hypodermis and extending to the endodermis is the mesophyll, which forms the bulk of the needle. The cells of the mesophyll are large, as compared with those of the other tissues, and contain the chloroplasts.

As seen in cross section, the mesophyll is composed of irregularly arranged, thin-walled cells (pl. 1, *A*). In the majority of the species, the mesophyll is not differentiated into spongy and palisade cells. Palisade cells were observed in *Picea breweriana*, *P. spinulosa*, *P. smithiana* Boiss. (syn. *P. morinda* Link), and *P. brachytyla* Pritzel. These cells, which are roughly rectangular in shape, are generally



A, Longitudinal section through the central portion of a needle of *Picea omorika* (Pancic.) Purkyne illustrating the arrangement of the mesophyll in layers separated by air spaces, × 75; *B*, longitudinal section of *P. sitchensis* near the endodermis showing the simple pits of the mesophyll cells, × 150.





A, Longitudinal section through the endodermis of *Picea breweriana*. Notice that the lateral walls have numerous simple pits in them, while the end or upper and lower walls are devoid of pits. × 400. *B*, Starch grains in the endodermal cells of *P. breweriana*. × 800.

arranged in one or two vertical layers along the sides of the needle adjacent to the hypodermis. The palisade cells are generally more abundant on the morphological lower side of the needle. This is the upper side when attached to the tree, owing to the twisting of the sterigmata. The spongy mesophyll cells are usually isodiametric, as in *P. pungens* Engelm., but they may be oblong or irregular in shape, as in *P. maximowiczii* Regel and *P. asperata*.

The cell walls of both the palisade and spongy mesophyll are slightly but uniformly thickened and, according to Feustel (*5*), strongly lignified. The walls in most species are smooth or slightly wavy but may be strongly infolded or corrugated as in *Picea breweriana*. The adjoining cells are in close contact with one another and usually without interstitial spaces. Interstitial spaces were observed only in *P. wilsonii* Masters, *P. orientalis* Carrière, *P. polita*, *P. sitchensis*, and *P. breweriana*.

The appearance of the longitudinal section is considerably different from that of the cross section. In the former the cells are flatly oblong, sometimes doubly concaved, and are arranged in uniseriate layers separated by air spaces varying in width (pl. 5, *A*).

Toward the hypodermis the layers become two or more cells thick and unite. The mesophyll is thus continuous along the hypodermis for a depth of two or three cells. This continuity of the mesophyll does not occur at the endodermis where the layers maintain their individuality.

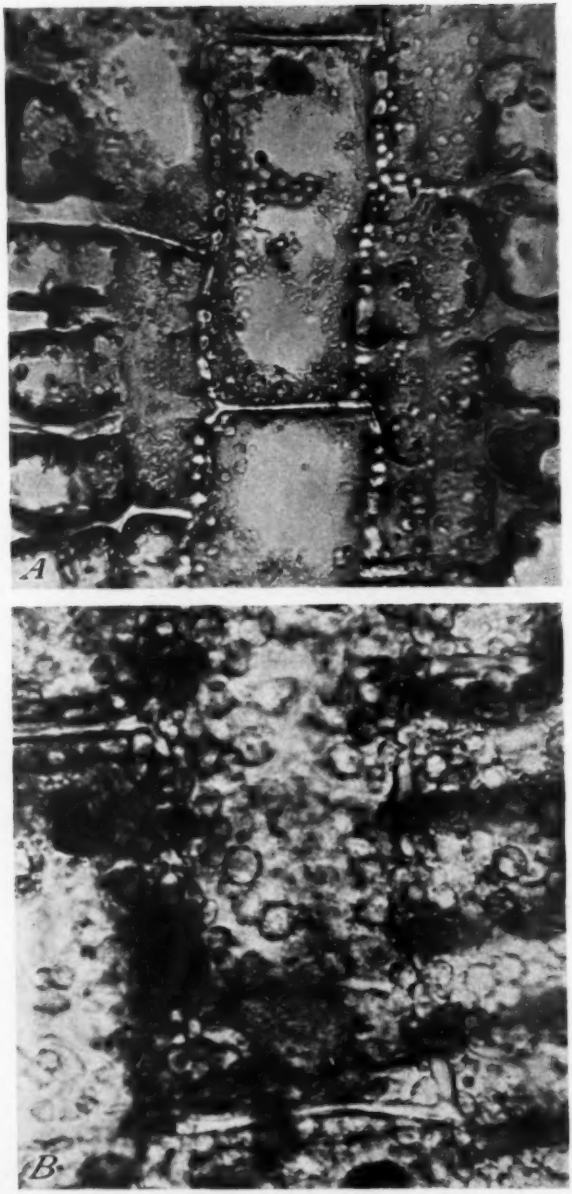
Longitudinal sections show that the mesophyll cells are abundantly pitted (pl. 5, *B*). The pits, which are of the simple type, occur in the lateral walls of the cells and rarely in the upper and lower walls except in the region of the hypodermis where the mesophyll layers meet. The pits are best observed in sections tangent to the pit fields, because the plastids that adhere closely to the cell walls obstruct the view of these structures in other sections. Besides the usual inclusions, the cells often contain rather large rhombohedral crystals. These crystals when viewed under polarized light frequently display a variety of colors, principally white, red, and blue.

THE ENDODERMIS

In the central portion of a needle cross section there is a uniseriate, chainlike ring of cells, which is called the endodermis (pl. 1, *B*). Actually, the endodermis is a cylindrical sheath of cells that extends nearly the full length of the needle and encloses the transfusion tissue.

The endodermis in cross section does not show any striking structural characters. However, there are certain features such as the uniformity of cell size and the thickness of the walls that are of distinct diagnostic value for the identification of the various species. For example, in *Picea breweriana*, the walls of the endodermal cells are uniformly thickened, but the cells are unequal in size; in *P. abies* Karst. (syn. *P. excelsa* Link), the outer walls are thicker than the inner, and the cells are equal in size; in *P. polita* the walls are uniformly thickened, and the cells are equal in size; and in *P. spinulosa* the cells are unequal in size and irregularly shaped, and the walls are uniformly thickened. Starch grains have been found in the endodermal cells (pl. 6, *B*).

Longitudinal sections show that the endodermal cells are usually rectangular in shape and arranged vertically in horizontal rows. The vertical, i. e., the tangential and radial, walls contain numerous



A, Longitudinal section through the endodermis of *Picea breweriana*. Notice that the lateral walls have numerous simple pits in them, while the end or upper and lower walls are devoid of pits. × 400. *B*, Starch grains in the endodermal cells of *P. breweriana*. × 800.

arranged in one or two vertical layers along the sides of the needle adjacent to the hypodermis. The palisade cells are generally more abundant on the morphological lower side of the needle. This is the upper side when attached to the tree, owing to the twisting of the sterigmata. The spongy mesophyll cells are usually isodiametric, as in *P. pungens* Engelm., but they may be oblong or irregular in shape, as in *P. maximowiczii* Regel and *P. asperata*.

The cell walls of both the palisade and spongy mesophyll are slightly but uniformly thickened and, according to Feustel (*5*), strongly lignified. The walls in most species are smooth or slightly wavy but may be strongly infolded or corrugated as in *Picea breweriana*. The adjoining cells are in close contact with one another and usually without interstitial spaces. Interstitial spaces were observed only in *P. wilsonii* Masters, *P. orientalis* Carrière, *P. polita*, *P. sitchensis*, and *P. breweriana*.

The appearance of the longitudinal section is considerably different from that of the cross section. In the former the cells are flatly oblong, sometimes doubly concaved, and are arranged in uniseriate layers separated by air spaces varying in width (pl. 5, *A*).

Toward the hypodermis the layers become two or more cells thick and unite. The mesophyll is thus continuous along the hypodermis for a depth of two or three cells. This continuity of the mesophyll does not occur at the endodermis where the layers maintain their individuality.

Longitudinal sections show that the mesophyll cells are abundantly pitted (pl. 5, *B*). The pits, which are of the simple type, occur in the lateral walls of the cells and rarely in the upper and lower walls except in the region of the hypodermis where the mesophyll layers meet. The pits are best observed in sections tangent to the pit fields, because the plastids that adhere closely to the cell walls obstruct the view of these structures in other sections. Besides the usual inclusions, the cells often contain rather large rhombohedral crystals. These crystals when viewed under polarized light frequently display a variety of colors, principally white, red, and blue.

THE ENDODERMIS

In the central portion of a needle cross section there is a uniseriate, chainlike ring of cells, which is called the endodermis (pl. 1, *B*). Actually, the endodermis is a cylindrical sheath of cells that extends nearly the full length of the needle and encloses the transfusion tissue.

The endodermis in cross section does not show any striking structural characters. However, there are certain features such as the uniformity of cell size and the thickness of the walls that are of distinct diagnostic value for the identification of the various species. For example, in *Picea breweriana*, the walls of the endodermal cells are uniformly thickened, but the cells are unequal in size; in *P. abies* Karst. (syn. *P. excelsa* Link), the outer walls are thicker than the inner, and the cells are equal in size; in *P. polita* the walls are uniformly thickened, and the cells are equal in size; and in *P. spinulosa* the cells are unequal in size and irregularly shaped, and the walls are uniformly thickened. Starch grains have been found in the endodermal cells (pl. 6, *B*).

Longitudinal sections show that the endodermal cells are usually rectangular in shape and arranged vertically in horizontal rows. The vertical, i. e., the tangential and radial, walls contain numerous

well-defined, simple pits. No pits are evident in the transverse, or end, walls (pl. 6, *A*). The pits in the material studied always led to adjacent cells and none were observed leading to air spaces. According to Soar (*14*) the walls of the endodermal cells are lignified. In addition, the transverse, or end, walls are heavily suberized. This suberization and lignification plays a definite role in the function of the endodermis, as will be discussed in more detail later.

THE VASCULAR TISSUE

Within the endodermis is located the transfusion tissue. This tissue can be conveniently divided into the fibrovascular bundle and transfusion cells (pl. 1, *B*).

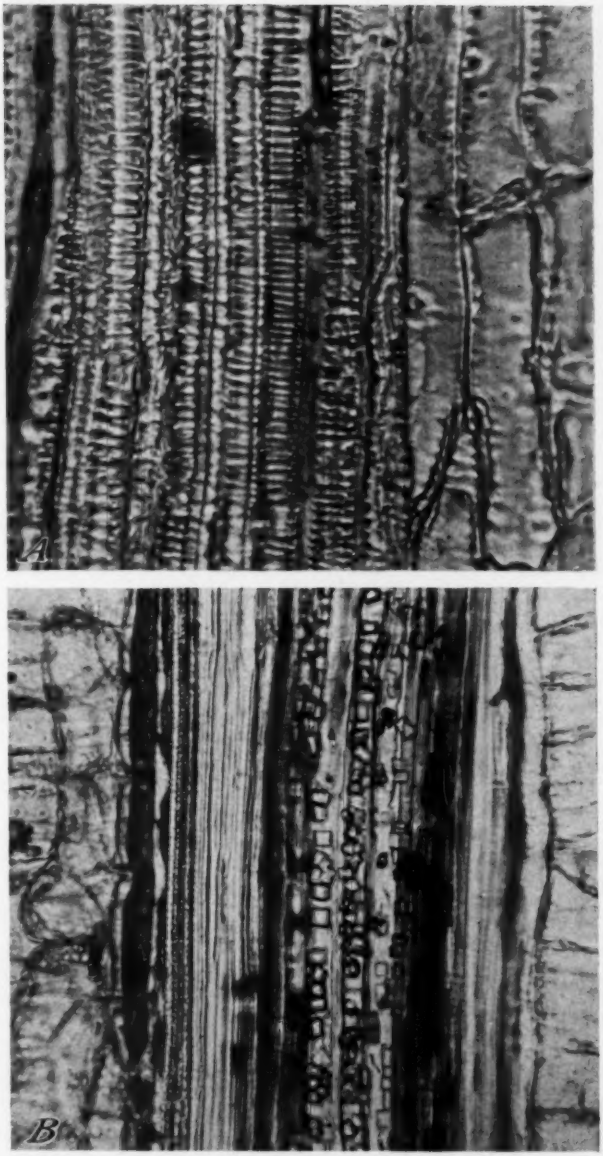
Fibrovascular Bundle

Although the spruces are generally credited with having but one fibrovascular bundle in their leaves, there is evidence that at an earlier evolutionary period there might have been two bundles. Lascassagne (*11*) states that in the cortex (sterigmata) the bundles of the leaf trace are distinct but in the leaves they are fused. In all species of spruce the bundles are distinctly divided, each part having an equal amount of xylem and phloem. Further, the phloem in many of the species is separated into two divergent wings.

The xylem forms the morphological upper side of the bundle and is composed of primary and secondary tracheids, parenchyma, and fibers. The primary tracheids are located at the lower margin of the xylem and comprise the first two or three rows. These cells have large bordered pits and distinct annular thickenings which may be closely or loosely arranged (pl. 7, *A*). The cells are short, thin-walled, and have distinct end walls. Generally each end wall contains a simple, large, bordered pit. The secondary tracheids are longer than the primary, thicker walled, and have long, tapering, overlapping ends. The cells are devoid of annular or spiral thickenings and have numerous bordered pits.

Xylem or wood parenchyma was found in all the species studied. It is generally arranged in uniseriate, vertical, raylike sheaths, which separate the bundles into equal parts (pl. 1, *A*). The cells, which are also arranged vertically, are short, oblong, and thick-walled and have numerous simple pits in their walls. Some observers who have studied only the cross section of the needles have reported medullary rays in the xylem, especially in those species with large bundles, such as *Picea breweriana* and *P. polita*. Study of the longitudinal sections shows that these rays are parenchyma sheaths similar to those described. The cells of the parenchyma sheaths frequently contain crystals (pl. 7, *B*).

The phloem also can be divided into primary and secondary. The primary phloem comprises two or three rows of cells along the lower margin of the bundle. The cells are compressed or crushed so that their lumina are not distinguishable. This crushing of the primary phloem seems to indicate that secondary growth takes place in the bundles. The secondary phloem cells are not as long as the tracheids and have well-defined lumina. The cells are thin-walled, usually blunt-ended, and have two to four sieve plates. They also have definite nuclei and cytoplasm. Companion cells were not observed.



A, Longitudinal section through bundle of *Picea breweriana* showing primary xylem with annular thickenings and secondary xylem with large border pits, × 800; *B*, longitudinal section through the center of the bundle of *P. breweriana* depicting the crystals that are frequently found in the phloem parenchyma cells, × 400.

Transfusion Cells

Within the endodermis and surrounding the fibrovascular bundle are the transfusion cells. These cells are of three types—parenchymatous, tracheal, and fibrous. The parenchymatous cells are nearly isodiametric, comparatively large, thin-walled, and simply pitted, and have conspicuous nuclei. The pits of these cells lead to similar cells, to cells of the phloem, or to endodermal cells. In the material studied, pits leading to the tracheal elements were uncommon. These parenchyma cells also contain oil globules, starch granules, and crystals.

The tracheal elements have thicker walls than the parenchymatous cells, and according to some authors, the walls are lignified. The cells are devoid of cell contents, are more or less rectangular, and have conspicuous bordered pits (pl. 3, *B*), which lead to other tracheal elements and to the endodermal cells.

The fibrous elements, or strengthening cells, are similar in all respects to the libriform fibers of the hypodermis. The fibers are long to very long with tapering end walls. The walls are very thick, lignified, and often display a laminated structure. The pits are simple and of the flattened, funnel-shaped type. These fibers are present in nearly all species above and adjacent to the phloem (pl. 1, *B*). In *Picea breweriana* and *P. brachytyla* the fibers are also found below the xylem. The abundance of these fibers can often be of use in the identification of the species. For example, in *Picea smithiana* they are usually 10 to 30 in number, while in *P. spinulosa* they are generally absent.

FUNCTIONS OF THE TISSUES

The functions of most of the leaf tissues are fairly clearly understood. It is generally recognized that the epidermis serves as protection; the stomata for regulating transpiration; the mesophyll for the elaboration of food products; and the fibrovascular bundles for the conduction of water and the translocation of the synthesized foods. On the other hand, the functions of the hypodermis, endodermis, and transfusion cells have not been clearly defined.

As already described, the hypodermis is composed of long to very long libriform fibers, which have very thick walls and long, tapering, overlapping ends. These cells are comparable to those found in wood, where their chief function is for strength and support. It is logical to assume that in the spruce needle also the primary function of these cells is for strength and support. Their secondary functions are for protection and, by virtue of their living cell contents, as intermediate agents for supplying the epidermal cells with the necessary nutrition materials. The fibrous elements adjacent to the resin canals serve in the same capacity, but those adjacent to the bundles must function purely for mechanical support, since living cell contents are not present in them.

Soar (*14*) intimates that the endodermis functions as a regulator for the passage of water to the mesophyll, since the radial and tangential walls are lignified and the transverse walls are suberized. This deduction is probably true, but the present study seems to indicate that water regulation may be a secondary function.

Leaves of hardwood trees have capillary veins which reach out into the mesophyll to collect and distribute evenly water and elaborated

foods. Frequently the capillary veins are reduced to a single tracheid, which is surrounded by specialized parenchyma or mesophyll cells. These specialized parenchyma or mesophyll cells function as intermediate agents for the collection and distribution of foods and water. In conifer leaves capillary veins are absent. Consequently, some tissue or group of cells must take over their function. The structure of the endodermis strongly suggests that one of its primary functions is the even distribution and collection of water and foods. As already mentioned, the fibrovascular bundles in the spruces are completely cut off from the mesophyll by the endodermis. Further, the endodermis is composed of specialized parenchyma cells, which are arranged in more or less horizontal rows. The cells in a given row are independent of the cells in the rows immediately above or below, because of the heavily suberized end walls devoid of pits. These facts, coupled with the layering of the mesophyll, indicate that a single row of endodermal cells insures the even distribution and collection of water and foods from the adjoining mesophyll layer.

The structure of the transfusion cells also indicates the passage of water from the fibrovascular bundle to the endodermis, and the return to the bundle of elaborated foods. The tracheal cells are lignified and have bordered pits—features typical of water-conducting elements. The tracheids communicate by means of their bordered pits with similar cells, with the endodermis,with the xylem of the bundle, and, rarely, with other cells. Hence, a tracheal strand figuratively forms a direct passage for water from the bundle to the endodermis. Similarly the parenchyma cells, which are characterized by living cell contents, unlignified walls, and simple pits, form the passageway for elaborated foods to get to the phloem from the endodermis. This hypothesis is substantiated by the fact that starch grains, crystals, and oil globules are common inclusions of the parenchyma cells, while they are entirely lacking in the tracheal cells.

The continuity of pitted cells from the fibrovascular bundle to the epidermis should be pointed out here. This continuity means that water and elaborated foods in solution pass from cell to cell by osmosis through the pit membranes and not through the semilignified cell walls as is commonly thought.

SUMMARY

The present study of spruce needles has revealed several interesting anatomical features, some of which have supplied the answers to a few much mooted questions. These features are as follows:

The epidermal cells are equipped with teeth which project into the cuticle. These teeth help to anchor the cuticle firmly into position.

The epidermal cells are locked together by a modified dovetailed joint.

The variation in the size of the lumina of the libriform hypodermal fibers is not of diagnostic value, since the fibers overlap.

The structure of the guard cells is unlike any heretofore described or portrayed. In particular, the thickened upper and lower walls of the guard cells in the region of the stoma are separated by two extremely thin, flexible membranes. When the stomata are opened, the membranes collapse and the two guard-cell walls come in contact.

All spruce needles have two longitudinal, parallel series of resin ducts or cysts. These ducts or cysts are usually located in the lateral

angles of the needles adjacent to the dermal tissue. In a given cross section no, one, or two ducts or cysts may be evident. Consequently, the number of ducts or cysts per section is not of diagnostic value. The diameters of these structures, however, are constant within certain limits and can be used in identification.

The mesophyll cells are arranged in uniseriate layers, separated by air spaces. The cells are equipped with rather large simple pits.

The endodermis is a uniseriate, cylindrical sheath of cells which separates the transfusion tissue from the mesophyll. By virtue of its pitting and the chemical nature of its cell walls the endodermis serves as a distributing and collecting agent for water and elaborated foods.

The transfusion cells are chiefly tracheal and parenchymatous. The two types of cells serve respectively in the conduction of water and elaborated foods.

The number of strengthening, or fibrous, cells adjacent to the bundles is of diagnostic value.

The spruces have two fibrovascular bundles in their petioles, which become fused into one in the needle. The bundle is separated vertically into two equal parts by a raylike sheath of parenchymatous cells.

LITERATURE CITED

(1) Bastin, Edson S., and Trimble, Henry.
1896–97. A contribution to the knowledge of some north american coniferæ. Amer. Jour. Pharm. 68: 21–39, 65–79, 136–140, 199–210, 242–254, 321–337, 383–386, 409–422, 554–566, 642–648, 1896; 69: 90–97, 354–359, 1897, illus.

(2) Burnet, Abbe Ovide.
1866. histoire des picea qui se rencontrent dans les limites du canada. 16 pp., illus. Quebec.

(3) Dorner, Herman B.
1900. the resin ducts and strengthening cells of abies and picea. Ind. Acad. Sci. Proc. 1899: 116–129, illus.

(4) Durrell, L. W.
1916. notes on some north american conifers based on leaf characters. Iowa Acad. Sci. Proc. 23: 519–582, illus.

(5) Feustel, Herm.
1921. anatomie und biologie der gymnospermblätter. Bot. Centbl. Beih. (II) 38: [177]–257.

(6) Fulling, Edmund H.
1934. identification, by leaf structure, of the species of abies cultivated in the united states. Bull. Torrey Bot. Club 61: 497–524, illus.

(7) Gauba, Erwin.
1926. beiträge zur biologischen anatomie des koniferblattes. Biologia Generalis 2: [301]–337, illus.

(8) Hannig, E.
1922. untersuchung über die harzbildung in konifernadeln. Ztschr. f. Bot. 14: [385]–421, illus.

(9) Harlow, W. M.
1931. the identification of the pines of the united states, native and introduced, by needle structure. N. Y. State Col. Forestry, Syracuse Univ., Tech. Pub. 32, 21 pp., illus.

(10) Hayata, B., and Satake, Y.
1929. contributions to the knowledge of systematic anatomy on some japanese plants. Bot. Mag. Tokyo 43: 73–106, illus. [In Japanese. Authors' names and title also given in English.]

(11) Lacassagne, Marcel.
1934. étude morphologique, anatomique et systématique du genre picea. Trav. Lab. Forestier Toulouse t. 2, (Études Dendrol. 3) Art. 1, 292 pp., illus.

(12) MARCO, H. F.
1931. NEEDLE STRUCTURE AS AN AID IN DISTINGUISHING COLORADO BLUE SPRUCE FROM ENGELMANN SPRUCE. Bot. Gaz. 92: 446–449, illus.
(13) SCHWENDENER, S.
1898. GESAMMELTE BOTANISCHE MITTHEILUNGEN. 2 v., illus. Berlin.
(14) SOAR ISABEL.
1922. THE STRUCTURE AND FUNCTION OF THE ENDODERMIS IN THE LEAVES OF THE ABIETINEAE. New Phytol. 21: 269–292, illus.
(15) SUTHERLAND, M.
1934. A MICROSCOPICAL STUDY OF THE STRUCTURE OF THE LEAVES OF THE GENUS PINUS. New Zeal. Inst. Trans. and Proc. 63: 517–568, illus.
(16) THOMAS, FRIEDRICH.
1865. ZUR VERGLEICHENDEN ANATOMIE DER CONIFEREN-LAUBBLÄTTER. Jahrb. Wiss. Bot. 4: 23–63.

INFLUENCE OF MOISTURE AND LOW TEMPERATURE ON THE GERMINATION OF HOP SEEDS[1]

By D. C. SMITH

Formerly *agent, Division of Drug and Related Plants, Bureau of Plant Industry, United States Department of Agriculture,* and *assistant agronomist, Oregon Agricultural Experiment Station*

INTRODUCTION

Though hop plants (*Humulus lupulus* L.) are propagated commercially by the use of cuttings from underground stems, breeding work requires the sexual propagation of the species. The writer became interested in the possibilities of low temperatures in stimulating the germination of hop seeds because of the relation of this problem to varietal improvement. Investigations in progress included the growing of seedlings for observation, selection, and determination of the results of controlled pollination with numerous individual plants. Germination of most seed lots was extremely low, ranging from 0 to 10 percent even after they had remained under apparently favorable germination conditions for several weeks. Seedlings developed from very few of the numerous seeds sown, and the number of plants from which to make selections was therefore small and the difficulties of studying the effects of controlled pollination were greatly increased. For these reasons experiments were undertaken, the results of which are presented here.

The present work is concerned with the germination aspects of treatments of hop seeds, including moisture and temperature factors. The term "dormancy" is used in a general sense, no attempt having been made to study the nature of the seed in its relation to germination. The investigations were made at Corvallis, Oreg.

REVIEW OF LITERATURE

In recent years much has been written concerning the effects of various treatments on the physiological behavior of seeds and plants. The results of subjecting dormant seeds or seeds in early stages of germination to controlled-moisture and low-temperature conditions have been studied both by those interested in germination and by others concerned principally with the effects on subsequent growth.

Crocker (*2*) [2] and Toole (*9*) presented discussions of the general aspects of seed dormancy and indicated certain factors as influential in hastening afterripening. Pertinent literature was also cited by these writers. Nichols (*6*) studied the influence of low temperatures on seed germination and found growth of certain native species to be hastened by exposure to cold. Mirov (*5*) reported that low-temperature treatments stimulated germination in certain coniferous species.

[1] Received for publication July 6, 1938. Cooperative investigation of the Division of Drug and Related Plants, Bureau of Plant Industry, U. S. Department of Agriculture, and the Oregon Agricultural Experiment Station.

[2] Italic numbers in parentheses refer to Literature Cited, p. 380.

Haut (*4*) found low-temperature storage at several temperatures to stimulate afterripening and promote germination of pear, apple, cherry, and peach seeds.

Raum (*7*), in a discussion of hop-improvement problems, directed attention to the difficulty of obtaining prompt and complete germination of hop seeds. Sprouting was found to be almost complete, however, if seeds were placed in pots and subjected to outdoor conditions during the winter until April. Bressman (*1*) referred to the use of chilling and scarification in stimulating hop seed germination. The treatment suggested was 10 days at freezing temperatures followed by scarification with a coarse emery cloth.

Though other workers in hop breeding have undoubtedly encountered the problem of initial low germination of hop seeds under certain methods of handling, no references to this subject other than those noted have been seen by the writer.

MATERIALS AND METHODS

In preliminary experiments, 1934, seeds of Late Clusters, Early Clusters, Fuggles, and Red Vine, principal hop varieties of the Pacific coast, were placed upon moist blotters in Petri dishes. Conditions for germination were kept favorable with respect to moisture, and temperatures ranged from 15° to 23° C. The dishes were kept in indirect light. A similar series was incubated in blotters in a standard germination chamber at room temperature. No pretreatments of any kind were given, but midway through the period the seeds and substratum became very moldy. The seeds and Petri dishes were then washed in a dilute solution of an organic mercury disinfectant, new blotters were supplied, and incubation was continued as before.

Incidental tests were made also with several periods of refrigeration at different low temperatures. Eight lots of seeds of each of the four varieties were placed in cold storage at 5° and −12° C. for periods of 2, 4, and 6 weeks at the former and 2 weeks at the latter temperature. The seeds were sown in soil at the termination of the periods, and observations were made on germination.

A more extensive experiment was completed in 1936. Lots of seeds of the four varieties previously studied were tested with four incubation periods before cold storage, five periods of refrigeration, and two temperatures. The combinations of these variables in treatment series comprised 40 tests of each variety, as listed in table 2. The tests were made in triplicate, and three check series were also included. Check 1 consisted of seed lots that received no wetting, incubation period, or cold storage but were sown, without any treatment, direct in the soil. Check 2 was not wetted or incubated in the germinator but was stored 2 weeks at −12° C. Check 3 was treated like check 2 except that the cold storage was at 5°.

Dry seeds were counted out in lots of 100 each. They were then placed in folded, moist paper toweling and incubated in a standard germinator for the periods indicated. Care was taken to avoid exclusion of air in folding the toweling. At the end of the incubation period the lots were immediately placed in refrigeration and left for the desired time. They were then removed, and the seeds were planted one-half inch deep in short rows in greenhouse benches. Lots

of the —12° C. series were allowed to thaw 24 hours at about 70° F. before seeding.

The term "incubation period," as used herein, refers to the period in the germinator during which no seeds germinated. Germination percentages were noted at intervals of 2 weeks from the date of seeding of each lot, percentages being recorded for 2, 4, 6, and 8 weeks after seeding.

In order to obtain further information on the variability of germination among seed lots from different plants, germination tests were made with seeds of the same crop year from 204 individual plants. All lots were first subjected to 2 weeks' refrigeration at 5° C. One series was then sown in field coldframes without bottom heat with outdoor temperatures ranging from 20° to 80° F. A second series was sown in greenhouse beds, with temperatures during germination ranging from 40° to 90° F. Material planted in coldframes was subject to seasonal rains; that in the greenhouse was artificially watered.

It is a common observation among growers that hop seedlings begin to appear in late January or February under field conditions in western Oregon. These develop from seeded hop cones that fall to the ground during harvest or remain on the vines when the latter are cut down following picking. The seeds evidently undergo a rest period in the field and begin to germinate in late winter. In the fall and winter of 1935–36 seeds were gathered September 25, November 25, and January 25 from the same individual plants in the field. The plants represented unnamed seedlings varying widely in morphological characters. After the collection of the last lot the samples of the three collections were seeded in greenhouse benches, and observations were made on germination. This test was not replicated.

In the experiments outlined, care was taken to include only sound seeds, since many apparent "seeds" are only hulls. Seeds of varieties and of individual plants varied in size to some extent. In threshing the seeds from the strobiles by hand the persistent perianth was not removed. No seeds germinated in the incubation period and none were observed to be markedly swelled by this treatment. Unless otherwise noted, 100 seeds were included in each lot.

The soil used in the cultures, both field and greenhouse, was a medium sandy loam of the Chehalis series, and no commercial or other fertilizer was applied. The soil was kept reasonably moist. Seeds were considered to be germinated when the seedlings were beginning the third leaf. No damping-off or other seedling disease was noted in any of the trials. During the period of germination greenhouse temperatures varied between 45° and 90° F., the most frequent range being from 60° to 80° F. The type of refrigeration used was that provided by a small ammonia plant with temperatures kept reasonably constant. No variations of more than 3° C. above or below the temperatures indicated were observed.

RESULTS

PRELIMINARY GERMINATION TESTS

Data for the preliminary tests of germination in blotters and Petri dishes are given in table 1. The figures represent additional seeds that germinated at each successive date. Since there were but 50

seeds in each lot, percentage may be derived by multiplying the figures by 2 in each instance. Though the Petri dishes were kept in daylight and the blotters in a standard germinator, it is obvious that even after long periods seeds may fail to sprout. Very few seeds softened during the period.

TABLE 1.—*Germination of hop seeds of several varieties in Petri dishes and germination blotters* [1]

Variety	Additional seeds germinated after indicated incubation period (days) in—																			
	Blotters										Petri dishes									
	15	20	25	30	35	45	50	60	70	75	15	20	25	30	35	45	50	60	70	75
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
Red Vine	4	1	0	0	0	0	1	0	0	0	1	2	1	0	0	0	0	0	0	0
	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fuggles	1	0	0	0	0	3	1	0	0	1	1	0	0	1	0	0	0	1	1	1
	0	0	0	1	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Late Clusters	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
	0	0	0	0	1	0	1	0	0	0	0	0	1	0	1	0	0	1	0	1
Early clusters	0	0	0	1	0	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

[1] 50 seeds were used in each test.

Additional preliminary data, derived from subjecting to low temperatures seeds from the same lots as those used in the blotter and Petri-dish tests, indicated that germination was modified favorably thereby. Though the trials were cursory, relative effects of refrigeration periods showed 6-, 4-, and 2-week periods at 5° C., 2 weeks at −12°, and the check to rank in the order named in germination percentage.

MOISTURE AND TEMPERATURE RELATIONS

Table 2 contains data secured from a more extensive study of the interrelation of varieties, periods of incubation before refrigeration, refrigeration periods, and refrigeration temperatures with germination. Though little attention is given to the germination data for other than the final germination date, the values for the earlier dates may be of interest and are therefore included. Each germination value in table 2 represents an average of three replications. Check 1 included seed lots without treatment; checks 2 and 3 were held for 2 weeks at −12° and 5° C., respectively.

In table 3 the averages of each treatment for the last date of observation have been recorded to facilitate comparisons.

In general, variations between replicates were large. Throughout the 40 experimental series, percentage germinations ranged from very low to fairly high values, as the data indicate. Inclusion of all series in a determination of a generalized error, such as the analysis of variance, was therefore inadvisable. It was possible, however, to analyze series 23 to 25, 28 to 30, 33 to 35, and 38 to 40 by such a method (*3*) without serious infringement on data limitations. The results so analyzed are for the eighth-week observations only.

TABLE 2.—*Effect of various periods of incubation and refrigeration (at −12° and 5° C.) on germination of 4 varieties of hops 2, 4, 6, and 8 weeks after seeding*

Series No.	Incubation in germinator	Refrigeration		Germination [1] in indicated number of weeks after seeding—															
		Duration	Temperature	Early Clusters				Late Clusters				Fuggles				Red Vine			
				2	4	6	8	2	4	6	8	2	4	6	8	2	4	6	8
	Days	*Weeks*	*°C.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
1	2	1	−12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.33	0.33	0.67	0.00	0.00	0.00	0.33
2	2	2	−12	.00	.00	.00	.33	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
3	2	3	−12	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.33	.33	.00	.33	.67	.67
4	2	4	−12	.00	.00	.67	.67	.00	1.67	2.33	2.33	.00	.67	1.00	1.00	.33	2.00	2.00	2.00
5	2	5	−12	.00	.00	.00	.67	.00	1.67	2.67	3.33	.33	.67	.67	1.33	.33	1.67	2.33	3.33
6	5	1	−12	.00	.00	.00	.33	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
7	5	2	−12	.00	.00	.00	.00	.00	.00	.33	.33	.00	.00	.00	.33	.00	.67	1.00	1.33
8	5	3	−12	.00	.00	.00	.00	.00	.00	.00	.33	.00	.00	.00	.00	.00	.33	1.33	1.33
9	5	4	−12	.00	.00	.33	1.33	.00	.00	.00	.33	.00	.33	.33	1.00	.00	.00	.00	2.67
10	5	5	−12	.00	.33	.33	2.00	.00	4.00	5.33	5.67	.33	.33	.67	.67	.00	3.33	6.00	9.67
11	10	1	−12	.00	.00	.00	.00	.33	.33	.33	.67	.00	.00	.00	.33	.33	.33	.67	1.00
12	10	2	−12	.33	.33	.67	.67	.00	.00	.33	.67	.00	.00	.00	.00	1.00	4.33	6.00	7.00
13	10	3	−12	.00	.33	.33	.33	.33	.33	.33	.33	.00	.00	.00	.00	.00	.33	1.33	1.33
14	10	4	−12	.00	.00	.00	.33	.00	.33	.67	.67	.00	.33	33	1.00	.33	.33	.33	1.67
15	10	5	−12	2.00	9.33	13.00	13.00	3.00	14.67	16.67	16.67	1.00	16.33	20.33	20.33	3.00	27.33	34.33	34.33
16	15	1	−12	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.67	1.00
17	15	2	−12	.00	.33	1.67	1.67	.00	.00	.00	.00	.00	.67	.67	.67	.33	1.00	1.00	1.00
18	15	3	−12	.33	14.33	24.00	24.33	.00	4.00	6.67	7.67	.00	7.67	19.67	24.33	.00	22.67	38.00	42.67
19	15	4	−12	.33	3.33	8.67	8.67	2.00	17.67	25.67	25.67	5.33	19.00	23.00	23.00	1.33	15.67	23.67	23.67
20	15	5	−12	2.67	15.00	20.33	20.33	.33	8.33	14.00	14.00	.33	4.33	14.33	14.33	4.33	19.67	23.00	23.00
21	2	1	5	.00	.00	.00	.00	2.00	2.67	3.00	3.67	.00	.00	.00	.33	.00	.33	1.00	2.00
22	2	2	5	4.67	5.00	5.00	5.00	13.33	15.33	16.33	16.33	.00	.00	.67	1.00	6.00	6.67	7.67	9.00
23	2	3	5	6.00	9.67	10.67	10.67	30.33	39.33	41.33	41.67	5.00	12.00	13.67	13.67	19.33	26.67	27.33	27.33
24	2	4	5	40.00	48.67	49.67	49.67	44.00	52.33	52.67	52.67	35.67	40.67	42.67	42.67	51.00	61.00	61.33	61.33
25	2	5	5	32.00	63.67	64.00	64.67	24.67	45.33	46.33	47.00	29.00	70.33	72.00	72.67	33.33	66.67	67.33	68.00
26	5	1	5	.67	.67	.67	1.33	4.00	5.33	5.33	5.33	.00	.00	.33	1.33	2.67	3.67	4.00	5.00
27	5	2	5	14.67	16.67	17.00	17.00	7.33	9.00	9.33	9.33	.00	3.33	4.00	4.33	11.33	12.33	12.67	13.00
28	5	3	5	13.33	18.67	19.67	19.67	3.67	10.00	10.67	10.67	8.00	12.33	14.33	15.00	1.00	6.00	6.67	7.67
29	5	4	5	.33	6.67	14.67	18.67	.67	8.33	11.67	13.00	.67	7.00	14.00	17.33	.67	6.33	12.67	13.33
30	5	5	5	58.00	77.00	78.33	81.33	60.00	67.00	67.33	69.00	42.33	80.67	81.33	81.67	58.00	68.33	68.33	68.33
31	10	1	5	.00	.00	.00	.33	1.67	1.67	1.67	2.00	.00	.00	.33	.33	1.33	1.33	1.33	2.33
32	10	2	5	13.00	17.67	19.67	20.00	16.33	22.33	23.67	23.67	1.00	5.33	8.33	9.00	14.67	22.67	23.00	23.33
33	10	3	5	21.67	30.00	32.00	32.00	26.67	36.67	37.67	37.67	29.00	38.00	39.67	39.67	21.33	31.67	34.33	34.33
34	10	4	5	9.33	25.33	26.00	26.33	25.00	45.33	46.00	46.33	18.00	32.67	34.00	34.00	25.67	48.33	49.33	49.67
35	10	5	5	23.33	58.67	61.33	61.33	41.33	57.00	57.33	57.33	46.67	62.00	63.00	63.00	30.00	48.67	51.00	51.00

[1] Each value is an average of 3 replications.

131451—39——5

TABLE 2.—*Effect of various periods of incubation and refrigeration (at −12° and 5° C.) on germination of 4 varieties of hops 2, 4, 6, and 8 weeks after seeding*—Continued

Series No.	Incubation in germinator	Refrigeration		Germination in indicated number of weeks after seeding—															
		Duration	Temperature	Early Clusters				Late Clusters				Fuggles				Red Vine			
				2	4	6	8	2	4	6	8	2	4	6	8	2	4	6	8
	Days	*Weeks*	*° C.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
36	15	1	5	0.00	0.33	0.67	0.67	0.67	1.33	1.67	1.67	0.00	0.33	0.33	0.33	1.67	1.67	2.33	2.33
37	15	2	5	5.33	7.00	7.33	7.33	17.00	18.33	19.00	19.00	1.00	3.00	3.00	3.00	13.33	14.67	14.67	14.67
38	15	3	5	.00	22.00	32.00	32.33	1.33	40.33	44.67	45.67	.33	35.00	38.67	39.67	2.67	30.67	35.33	36.67
39	15	4	5	19.33	41.33	41.33	41.33	23.67	48.00	48.33	48.33	38.67	55.67	55.00	55.00	22.67	45.33	46.33	46.33
40	15	5	5	24.67	46.00	48.00	48.00	21.67	46.67	47.00	47.00	12.67	36.00	37.67	37.67	27.67	44.33	44.67	44.67
Check:																			
1 [2]				.00	2.00	2.00	2.33	.00	2.33	6.33	7.33	.00	.00	.00	.33	.33	.67	5.67	6.33
2 [3]				.00	.67	1.67	1.67	.67	1.67	3.33	3.33	.00	.67	1.33	1.67	14.33	16.00	17.00	17.00
3 [4]				.33	.67	1.33	1.67	.00	.67	1.33	2.00	.00	.33	.67	1.33	1.33	2.67	3.00	4.00

[2] Seeds received no treatment. [3] Seeds were stored 2 weeks at −12° C. without other treatment. [4] Seeds were stored 2 weeks at 5° C. without other treatment.

TABLE 3.—*Mean germination percentages of three tests for varieties, for incubation and refrigeration periods, and for temperatures of refrigeration*

Variety	Incubation in germinator	Germination after indicated refrigeration period (weeks) at specified temperature									
		1		2		3		4		5	
		−12° C.	5° C.	−12° C.	5° C.	−12° C.	5° C.	−12° C.	5° C.	−12° C.	5° C.
	Days	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
Early Clusters	2	0.0	0.0	0.3	5.0	0.0	10.7	0.7	49.7	0.7	64.7
Late Clusters	2	.0	3.7	.0	16.3	.0	41.7	2.3	52.7	3.3	47.0
Fuggles	2	.7	.3	.0	1.0	.3	13.7	1.0	42.7	1.3	72.7
Red Vine	2	.3	2.0	.0	9.0	.7	27.3	2.0	61.3	3.3	68.0
Average		.25	1.50	.08	7.80	.25	23.35	1.50	51.60	2.15	63.10
Early Clusters	5	.3	1.3	.0	17.0	.0	19.7	1.3	18.7	2.0	81.3
Late Clusters	5	.0	5.3	.3	9.3	.3	10.7	.3	13.0	5.7	69.0
Fuggles	5	.0	1.3	.3	4.3	.0	15.0	1.0	17.3	.7	81.7
Red Vine	5	.0	5.0	1.3	13.0	1.3	7.7	2.7	13.3	9.7	68.3
Average		.08	3.23	.48	10.90	.40	13.28	1.33	15.58	4.53	75.08
Early Clusters	10	.0	.3	.7	20.0	.3	32.0	.3	26.3	13.0	61.3
Late Clusters	10	.7	2.0	.7	23.7	.3	37.7	.7	46.3	16.7	57.3
Fuggles	10	.3	.3	.0	9.0	.0	39.7	1.0	34.0	20.3	63.0
Red Vine	10	1.0	2.3	7.0	23.3	1.3	34.3	1.7	49.7	34.3	51.0
Average		.50	1.23	2.10	19.00	.48	35.93	.93	39.08	21.08	58.15
Early Clusters	15	.0	.7	1.7	7.3	24.3	32.3	8.7	41.3	20.3	48.0
Late Clusters	15	.0	1.7	.0	19.0	7.7	45.7	25.7	48.3	14.0	47.0
Fuggles	15	.0	.3	.7	3.0	24.3	39.7	23.0	56.0	14.3	37.7
Red Vine	15	1.0	2.3	1.0	14.7	42.7	36.7	23.7	46.3	23.0	44.7
Average		.25	1.25	.85	11.00	24.75	38.60	20.28	47.98	17.90	44.35

The results of the use of the analysis of variance are shown in table 4. The data indicate significant differences to exist among refrigeration and incubation periods. The first-order interaction between refrigeration and incubation periods is also significant. Though the comparison of temperatures of refrigeration was necessarily omitted from the analysis, reference to table 2 and comparison of series 1 to 20 with series 21 to 40 make clear at once that a highly significant difference exists. Varietal differences were not significant.

TABLE 4.—*Analysis of variance data on varieties and incubation and refrigeration periods*

Variation due to—	Degrees of freedom	Sums of squares	Mean square	*F* [1]
Refrigeration periods	2	26,123	13,062	[2] 96.0
Incubation periods	3	2,825	942	[2] 6.9
Varieties	3	141	47	2.9
Refrigeration×incubation	6	17,412	2,902	[2] 21.3
Refrigeration×varieties	6	1,716	286	2.1
Varieties×incubation	9	1,628	181	1.3
Varieties×incubation×refrigeration	18	3,051	170	1.3
Error	96	13,092	136	
Total	143	65,986		

[1] Snedecor (8).

[2] Exceeds 0.01 point.

The standard error of a single analysis is 11.66 percent; the standard error of a mean of three tests, 6.73 percent. Since variety differences

were not significant, data for all varieties have been grouped in table 5 for comparison of germination and refrigeration periods. The minimum significant difference for refrigeration general means, based upon the 0.05 point, is 4.75 percent. The 3-, 4-, and 5-week periods, therefore, differ significantly in average germination. For comparing incubation-period means, the least significant difference is 5.50 percent. Accordingly, average germination was significantly lower following 5 days' incubation for all series. It should be borne in mind that these errors apply only to the series included in the analysis of variance.

TABLE 5.—*Mean percentage germination for all varieties for different refrigeration and incubation periods*

Incubation period (days)	Germination after refrigeration for—			Average germination
	3 weeks	4 weeks	5 weeks	
	Percent	*Percent*	*Percent*	*Percent*
2	23.4	51.6	63.1	46.0
5	13.3	15.6	75.1	34.7
10	35.9	39.1	58.2	44.4
15	38.6	48.0	44.4	43.7
Average	27.8	38.6	60.2	42.2

While the check series are not included in the calculation of error, most treatments show unquestionably significant increases in germination when compared with the check lots. The high germination of the Red Vine variety in check 2 is inexplicable.

Within refrigeration and incubation periods he least significant difference, twice the standard error of a difference, is 9.52 percent. On the basis of this error, incubation periods may be compared in relation to the several periods of refrigeration.

Considering lots incubated 2 days in the germinator and refrigerated at 5° C., those left for 4 weeks at low temperatures showed significantly higher germination than those refrigerated for 3 weeks, as an average of all varieties. Lots refrigerated 5 weeks were significantly higher in germination than those remaining at low temperatures for 3 weeks.

The superiority of longer periods of refrigeration following additional days in the germinator was not so marked. Comparing lots incubated in the germinator 10 days before chilling, 5 weeks' refrigeration was better than 4. Among lots incubated in the germinator 15 days before cold storage, refrigeration for 4 weeks was significantly superior to 3 weeks' treatment.

In the lots refrigerated 3 weeks, those incubated 10 and 15 days were significantly higher in germination than those incubated 2 or 5 days. Groups incubated 2 days were also significantly higher than the 5-day incubated groups.

Five-day incubated lots were significantly lower than 2-, 10-, or 15-day lots when refrigerated 4 weeks. The 2-day lot was significantly higher than that incubated 10 days.

When the lots refrigerated 5 weeks after different incubation periods are considered, additional relations are noted. Two-day incubation lots were superior to the 15-day series. Five-day incubation lots were significantly superior to all other groups when refrigerated for 5 weeks. Ten days' incubation was significantly better than 15

days. Fifteen-day incubation groups were significantly lower than others.

The value of F also indicated a significant interaction between incubation and refrigeration periods. Examination of the data in table 5 shows that in lots refrigerated 3 weeks, long periods of incubation were followed by increased germination, while with 5 weeks' refrigeration long incubation periods resulted in decreased germination. Using the extremes as an example,

$$(23.4-63.1)-(38.6-44.4)=-33.9$$

and the least significant difference in this comparison is 13.46 percent when based on twice the standard error of the difference. Since the trends of the differences are not closely consistent, it is possible that uncontrolled factors may have influenced the results.

Considering grouped averages of the entire 40 series, certain generalizations may be made. The greatest increase in germination occurred between the second and fourth weeks after seeding. Lots refrigerated at 5° C. showed a much faster germination rate the first 2 weeks and a somewhat more rapid progress the third and fourth weeks than those stored at −12°. It should be noted that some of the seeds sprouted during the fifth week at 5°, before removal from refrigeration. Most seeds germinating in all lots did so by the end of 4 weeks, with only slight increases thereafter.

Successive weeks in cold storage at either temperature resulted in consistent increases in germination, with no tendency toward decrease indicated, except in series remaining 15 days in the germination chamber. Five weeks at −12° C. gave results very similar to those obtained from storage for 2 weeks at 5°. Seed lots stored at −12° showed consistently increased germination as the periods of incubation were prolonged, and no tendency to decrease is indicated.

COMPARISON OF GERMINATION IN GREENHOUSE AND COLDFRAMES

Germination percentages on 40 lots of seed from individual plants seeded in greenhouse beds and field coldframes are listed in table 6. These are a random sample of 204 lots studied and are listed to indicate plant variations and germination rates. These data, though unreplicated, indicate wide variations in both rapidity and amount of germination in lots of seed from different plants. Many of these differences are undoubtedly significant. Since seed was collected only when the cones were well browned and dry, differences in maturity would appear to be of little consequence in explaining variation among plants.

Average germination for 204 lots in the greenhouse at the end of 60 days was 21.17 ± 0.87 percent, while that in coldframes was 18.85 ±0.85 percent. The P value for the difference does not quite reach the 0.05 point. The coefficient of correlation between percentages at 60 days, based on 196 plant lots, was 0.19. The P value, according to Fisher's t test (*3*), exceeds the 0.01 point. The slight average increase in greenhouse-germinated seed may have been due to the higher temperatures existing, better controlled moisture conditions, or other factors. There are instances listed where the field-planted seeds greatly exceeded in germination the same lots seeded in the greenhouse.

TABLE 6.—*Germination percentages of seed lots from individual plants sown in greenhouse or field after 14 days' incubation in moist blotters followed by 14 days' refrigeration at 5° C.*

Plant No.	Germination in indicated number of days after seeding in—					Plant No.	Germination in indicated number of days after seeding in—				
	Greenhouse			Field			Greenhouse			Field	
	15	25	40	60	60		15	25	40	60	60
	Pct.	Pct.	Pct.	Pct.	Pct.		Pct.	Pct.	Pct.	Pct.	Pct.
2-4	0	3	9	9	6	25-2	3	5	6	14	41
2-33	7	18	26	30	31	25-3	0	2	5	9	24
4-31	7	18	25	32	16	25-4	2	6	9	17	13
4-33	39	41	41	48	28	25-5	9	20	21	23	6
5-32	12	13	14	25	15	26-1	21	30	35	36	21
6-32	20	29	29	36	33	26-2	4	5	8	10	7
8-10	8	16	18	18	43	26-12	5	10	10	19	15
8-12	19	30	33	33	33	27-3	8	16	18	19	12
9-7	5	14	15	19	9	27-12	13	25	28	28	37
15-32	9	13	15	16	37	27-31	8	17	21	22	10
18-31	16	26	29	29	22	29-3	17	33	44	46	34
19-5	9	14	16	16	24	29-4	22	25	25	25	14
19-13	14	27	34	34	36	29-34	6	11	28	28	19
20-13	6	13	17	21	6	32-4	7	11	20	20	11
20-33	0	3	4	12	7	32-10	10	14	15	15	39
22-10	5	14	17	21	32	35-5	6	10	11	20	18
25-30	9	12	17	22	32	37-4	11	18	21	23	7
24-4	10	25	27	30	28	37-18	6	11	14	14	29
24-32	5	8	15	19	24	37-22	0	4	9	18	5
24-34	1	1	2	17	21	38-4	0	6	8	12	42

EFFECT OF NATURAL CONDITIONS ON STIMULATION OF GERMINATION

Data obtained concerning the effects of weathering on the germination of 16 single-plant seed lots are given in table 7. September 25, the first date of collection, represents a period when the seeds might be considered as nearly mature and browning of cones well started. The November and January collections represent seeds from cones which, with the vines, had fallen to the ground and remained in proximity to the soil for approximately 50 and 110 days respectively, being subject to various weather factors. Pertinent phases of the climatic data for the period during which seeds weathered in the field are given in table 8. These may be of further interest in considering the data of germination.

TABLE 7.—*Percentages of germination of single-plant seed lots allowed to remain in the field for 2 periods following maturity* [1]

Plant No.	Germination, in indicated number of days after sowing, of seeds collected—														
	Sept. 25					Nov. 25					Jan. 25				
	10	25	40	55	70	10	25	40	55	70	5	10	25	35	50
	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.
2-4	0	0	0	0	0	6	16	16	16	17	26	43	44	44	45
8-12	0	0	0	0	0	0	3	3	3	3	6	11	16	16	16
15-32	0	0	0	0	0	0	1	2	2	2	7	12	26	26	26
18-31	0	1	1	1	2	14	26	26	26	27	2	9	21	23	23
19-5	0	1	1	1	1	1	8	9	9	12	25	37	45	45	46
20-33	0	0	0	0	0	6	12	12	12	12	----	----	----	----	----
22-10	0	0	0	0	0	0	1	1	2	2	11	16	22	23	23
25-4	0	0	0	1	1	2	3	3	4	5	12	16	24	27	28
26-12	0	0	0	0	1	3	4	4	4	4	5	12	13	15	16
27-3	0	0	0	0	0	5	9	9	9	10	10	32	43	43	44
27-31	0	0	0	0	0	2	2	2	2	2	8	9	10	10	11
29-4	0	1	1	1	0	0	9	11	11	11	13	16	21	22	23
38-31	0	0	0	0	0	0	2	2	2	2	7	15	28	29	29
40-33	0	0	0	0	0	0	0	0	0	0	15	31	33	34	34
45-32	0	0	0	0	0	0	0	0	0	0	3	7	24	27	28
47-32	0	0	0	0	0	0	2	2	3	4	6	11	16	19	20
Average	0	0.2	0.2	0.3	0.3	2.4	6.1	6.4	6.6	7.1	10.4	18.5	25.7	26.9	27.5

[1] Data based on 100 seeds of each sown in greenhouse benches.

TABLE 8.—*Summary of weather data for September to December 1935, and January 1936, at Corvallis, Oreg.*

Month	Precipitation		Temperature			
			Maximum		Minimum	
			Mean	Range	Mean	Range
	Days	*Inches*	*° F.*	*° F.*	*° F.*	*° F.*
September	2	1.36	81.7	69–97	51.3	43–59
October	12	[1] 1.95	63.1	36–84	42.6	30–57
November	12	2.26	49.9	36–59	35.5	15–46
December	15	4.76	46.8	39–58	34.3	24–46
January	19	10.82	49.9	44–58	38.1	24–46

[1] 5 inches of snow Oct. 31 not included.

The results show the natural increase in germinating capacity of seeds left to weather in the field and demonstrate the collective influence of the various factors in overcoming dormancy under field conditions. Again variation between lots of seeds from different plants may be noted. In two lots only did seeds collected November 25 fail to germinate better than those obtained September 25, though some differences are small. Seeds gathered January 25 germinated better than those collected November 25 in all lots except one. Seeds from plants 40–33 and 45–32 required longer exposures in the field to induce germination.

DISCUSSION

The results of the experiments have shown that subjection of hop seeds to certain low-temperature treatments exerts a marked beneficial influence on germination percentage. From the data presented it seems probable that longer periods of incubation before storage at −12° C. would have stimulated germination still more. The length of the incubation period seemed to be unrelated to germination following storage at 5°. It also seems likely that periods of refrigeration longer than 5 weeks might have resulted in increased germination.

In the experiment concerned with moisture and temperature treatments, considerable variation in germination existed among varieties. While significant differences were not established, it appears probable that the Red Vine variety might have reacted somewhat differently from the other strains, particularly at storage temperatures of −12° C. More replications would have been desirable to establish variety differences.

The data of table 6 are of special interest in showing wide variations in the germinating habits of seeds from different plants. It should be recalled that hop plants are dioecious. Since the pollination process was uncontrolled, widely different males may have contributed to seed formation in the numerous female plants. Correlation of germination percentages of seed lots from the same plants from year to year would serve to further delineate this character. While treatment of seeds by germinator incubation for 5 days, followed by 5 weeks' storage at 5° C., appeared to be the best of the series used in these experiments, certain seed lots from various female seedlings used in breeding might respond better to other conditions.

From the standpoint of progress in breeding investigations, subjecting seeds derived from controlled pollination to periods of low-temperature storage shortly after maturity allows for greater latitude in utilization and study and more careful control of the material than awaiting germination out of doors. Germination in the former instance may also be more rapid and more complete.

The effects of the treatments on growth after germination were not carefully observed. Differences between lots were probably slight, since the seedlings appeared to be extremely uniform in this respect as growth progressed.

SUMMARY

Germination is very low when recently harvested hop seeds are placed under ordinary conditions of moisture and temperature such as are favorable to the germination of seeds of many plants. Hop seeds undergo a period of dormancy upon maturity of the cone, which, under natural conditions, may be overcome by interaction of weather factors. Seeds from individual plants vary greatly in rate and total amount of germination both under natural conditions and after artificial treatments.

No significant differences were established in reaction among varieties.

The relations of moisture, temperature, and variety to seed germination have been studied. Five days' incubation in the germinator followed by 5 weeks' refrigeration at 5° C. gave the highest germination in the treatments used. Many treatment series exceeded the check lots significantly.

In these experiments storage of moist seeds at 5° C. was greatly superior to storage at −12° in stimulating germination. Three-, four-, and five-week storage periods at 5° resulted in significantly increased germination as the length of the period increased.

Generally longer periods of incubation were accompanied by increased germination in the series refrigerated at −12° C. In the effects upon germination, interrelation of periods of incubation in the germinator and length of the subsequent refrigeration periods at 5° was indicated.

In the general study of incubation and refrigeration periods and temperatures, most germination occurred between 2 and 4 weeks after seeding, regardless of treatment. Hop seeds sprouted at 5° C. after 4 weeks' refrigeration at that temperature.

Total germination of seed lots of 196 plants after 2 weeks' refrigeration at 5° C. and after 60 days in field and greenhouse was significantly correlated and not significantly different.

LITERATURE CITED

(1) Bressman, E. N.
1931. developing new varieties of hops. Science 74: 202–203.

(2) Crocker, Wm.
1925. points of agronomic interest in the physiology of germination. Jour. Amer. Soc. Agron. 17: 696–705.

(3) Fisher, R. A.
1936. statistical methods for research workers. Ed. 6, rev. and enl., 339 pp., illus. Edinburgh and London.

(4) Haut, I. C.
1934. the effect of various low temperatures upon the afterripening of fruit tree seeds. Amer. Soc. Hort. Sci. Proc. (1933) 30: 365–367.

(5) Mirov, N. T.
1936. A NOTE ON GERMINATION METHODS FOR CONIFEROUS SPECIES. Jour. Forestry 34: 719–723.
(6) Nichols, G. E.
1934. THE INFLUENCE OF EXPOSURE TO WINTER TEMPERATURES UPON SEED GERMINATION IN VARIOUS NATIVE AMERICAN PLANTS. Ecology 15: 364–373.
(7) Raum, H.
1929. ÜBER SORTENWESEN IM BAYERISCHEN HOPFENBAU UND WEGE DER HOPFENZÜCHTUNG. Fortschr. Landw. 4: 342–345.
(8) Snedecor, George W.
1934. CALCULATION AND INTERPRETATION OF ANALYSIS OF VARIANCE AND COVARIANCE. 96 pp. Ames, Iowa. (Iowa State Col. Agr., Div. Indus. Sci. Monog. 1.)
(9) Toole, E. H.
1936. PHYSIOLOGICAL PROBLEMS INVOLVED IN SEED DORMANCY. Internatl. Seed Testing Assoc. Proc. 8: 33–41.

INHERITANCE OF EGGSHELL THICKNESS IN WHITE LEGHORN PULLETS[1]

By LEWIS W. TAYLOR, *head, division of poultry husbandry, and* I. MICHAEL LERNER, *junior poultry husbandman, California Agricultural Experiment Station* [2]

INTRODUCTION

Evidence that individual hens differ with regard to eggshell expressed as a percentage of total egg weight has been available at least since Willard and Shaw (*18*)[3] published data on eggs from Barred Plymouth Rocks, White Leghorns, White Wyandottes, and American Reds. These writers, however, dealt with an insufficient number of birds to establish breed differences with respect to the composition of eggs. Herrasti (*6*) reported brown-shelled eggs to have a higher breaking strength than white-shelled eggs, but the source of the eggs studied was not given, and the method used was inexact. Significant, high correlations between breaking strength and thickness or percentage of shell have since been conclusively established by Romanoff (*14*), Morgan (*9*), and Stewart (*15*). Romanoff also produced evidence of individual bird differences in breaking strength of shells from White Leghorn pullets.

Taylor and Martin (*16*) published data indicating that the ability to form a thick eggshell was controlled in part by hereditary factors. Eggs from a Barred Plymouth Rock flock were significantly lower in percentage of shell than eggs from a White Leghorn flock. Within the White Leghorn flock, families of daughters from two males differed significantly in the percentage of shell in their eggs. Morgan, Mitchell, and Roderick (*11*) and Morgan (*9*), incidental to other considerations, presented data on the percentage of shell in eggs of Barred Plymouth Rock and White Leghorn flocks. Although these writers drew no conclusion in this respect, their data indicate that the flocks of the two breeds used were significantly different with respect to percentage of shell and breaking strength of shell.

Baskett, Dryden, and Hale (*2*) found that the individuality of the hen was the most important factor in determining the breaking strength of the eggshells produced, although no significant differences between White Wyandottes, White Leghorns, and Rhode Island Reds were found. A definite seasonal effect on breaking strength observed was relatively less important. Munro (*12*) has recently published an analysis of a Barred Plymouth Rock flock which shows significantly higher variance between the offspring of different dams than within the progeny of individual dams in the characters of percentage ash of dry shell and percentage ash of total egg. The differences between groups of daughters from individual dams are attributed to differences in genotype.

[1] Received for publication July 15, 1938.
[2] Acknowledgement is due C. A. Gunns, who was responsible for the collection of the basic data. Statistical assistance was provided by the Works Progress Administration.
[3] Italic numbers in parentheses refer to Literature Cited, p. 396.

EXPERIMENTAL PROCEDURE

ORIGIN OF SELECTED LINES

In 1932 a preliminary investigation of shell thickness produced by Single-Comb White Leghorn hens of the California Station flock revealed that certain related groups of birds were more uniform with respect to this character than others.

Three full sisters mated to a half brother (band No. F15) in a study of another egg character were found to produce thin-shelled eggs. An older half sister of the above females (common dam) mated to a different and nonrelated male (band No. F19) also produced thin-shelled eggs. These matings were then designated as thin-shelled selections for 1932. Only five female offspring were produced in that year. In 1933 the surviving dams with their daughters and additional related thin-shelled females were mated to a son from one of the three original full sisters and her half brother. In subsequent years no other stock was added in propagating this line, which for the sake of brevity will henceforth be referred to as the thin line.

Also in 1932 a half brother (common sire) of the thin-shelled male F19, was mated to his dam, his three full sisters, and to seven females not related to him but producing thick-shelled eggs. This formed the original thick-shelled mating, and offspring from it will be referred to as the thick line. In 1933 a cockerel from the thick-shelled line mated to his eight full sisters formed the thick-line selection. As in the case of the thin-shelled line, no other stock was added to the thick line in subsequent years.

BREEDING METHODS

TABLE 1.—*Percentage of shell for eggs of breeders and of their offspring in the successive generations*

Generation	Line	Number of birds and shell percentage for—									
		Dams			Daughters				Daughters used as breeders		
		Number	Mean	Range	Number	Mean	Range	Coefficient of variation	Number	Mean	Range
G	Thick	10	9.53	8.65–10.09	37	9.85	8.70–11.02	7.2	8	9.41	8.18–10.29
	Thin	3	7.97	7.27– 8.86	5	9.14	8.55–10.36	6.9	4	8.90	8.76– 9.37
H	Thick	8	9.41	8.18–10.29	38	9.84	9.12–10.72	4.2	6	10.05	9.62–10.34
	Thin	8	8.55	7.61– 9.37	27	9.44	8.65–10.30	4.9	5	8.77	8.65– 8.98
J	Thick	8	9.95	9.44–10.34	36	9.94	8.60–10.94	5.6	7	10.14	9.65–10.59
	Thin	7	8.81	8.65– 9.08	26	9.24	8.12–10.28	5.7	4	8.88	8.18– 9.31
	Thick ♀×thin ♂	2	10.16	10.00–10.32	6	9.65	8.63–10.11	5.8	5	9.56	8.63–10.11
K	Thick	9	10.10	9.62–10.59	28	9.79	8.72–11.15	5.5	.....	.....	.....
	Thin	5	8.85	8.18– 9.31	25	9.20	8.38–10.31	5.5	.....	.....	.....
	Thin ♀×thick ♂	5	9.00	8.69– 9.24	21	9.55	8.38–10.34	5.1	.....	.....	.....
	Thick ♀×thin ♂	5	10.45	9.82–10.94	37	9.91	8.93–10.91	4.4	.....	.....	.....
	Thin ♂ Thick ♀×thin ♂	5	9.56	8.63–10.11	37	9.51	8.19–10.63	7.2	.....	.....	.....

In subsequent years full brother by sister inbreeding was avoided wherever possible in mating both thick and thin lines. Families of daughters producing respectively thick- or thin-shelled eggs were mated to their half brothers or to their sires. Throughout the course of the experiment families showing great variability in the shell thick-

ness of the daughters were eliminated in the selection of breeders for further matings. The mean and the range of shell percentage for each year's matings in both thick- and thin-shelled lines are given in table 1. During 1934 and 1935 in producing the J and K generations, reciprocal crosses were made between the thick- and thin-shelled lines, and in 1935 a backcross of F_1 daughters was made to thin-shelled-line males.

In the progress of this experiment considerable difficulty was experienced in maintaining reasonable vigor under the conditions of inbreeding involved. Hatchability was maintained at a fairly satisfactory level, but rearing mortality was excessive in the last 2 years reported. The average coefficients of inbreeding of the daughters produced in the successive generations reported, expressed in terms of percentage, are shown in table 2.

TABLE 2.—*Average coefficients of inbreeding of daughters produced in successive generations of thick- and thin-shelled lines*

Generation	Thick-shelled	Thin-shelled
	Percent	*Percent*
G	9.84	10.00
H	18.75	7.01
J	26.85	20.94
K	30.08	28.33

DETERMINATION OF SHELL CHARACTERS

A uniform procedure was used throughout the experiment in obtaining data on shell thickness and on percentage of shell. Beginning in December and extending through January and February, eggs laid by all pullets in the selected lines were weighed and the shell and shell membranes removed and dried for 24 hours at 100° C. and then weighed. The shell thickness was measured by a micrometer at four or more points ranging from the blunt to the pointed end of the egg, the average value being recorded in terms of hundredths of a millimeter. All the birds from both lines were maintained each year in the same houses, subject to the same management. They were given access to the same ration and kind of supplemental calcium as well as to the same conditions of sunshine. A sample of 10 eggs from each bird was used to calculate average individual percentage of shell and shell thickness. This was found to be an adequate measure of the individual phenotypes, since analyses of variance for each character studied showed uniformly significantly greater variance between means of individuals than between the eggs produced by each individual. Minor variations were introduced in the rations fed from year to year, but in no instance did this factor play any determinable role with regard to the character of shell produced.

STATISTICAL METHODS OF ANALYSIS

Data obtained in the course of the 4 years of study were subjected to a series of statistical analyses. With regard to these, five points in the procedure should be explained.

1. The relation between the amount of shell and egg size can be expressed in several ways. The most common is the percentage of shell or the weight of the shell per unit weight of the whole egg.

Another method, proposed by Hendricks, Lee, and Godfrey (*5*), is that the amount of shell be measured with respect to the unit area of the egg surface, rather than its weight. An index of shell thickness on this basis would be given by the ratio of shell weight to the two-thirds power of the weight of the egg less the weight of the shell. This method is extremely laborious in application when individual eggs rather than means of groups of eggs are to be considered.

A third possible method is one that takes advantage of the allometric relation between a part and the whole, expressed in the formula $y=bx^{\alpha}$. In the terminology of Huxley and Teissier (*7*) α is the limiting equilibrium constant, determining the total amount of shell in an egg of a given size. The principle underlying this method may be interpreted in the same manner as that of the shell-thickness index of Hendricks, Lee, and Godfrey. The essential difference between these methods lies in the fact that while the latter proposes a functional relation between two parts of the whole, the ordinary allometric equation involves the relation between one part and the whole. The error introduced in the ordinary allometric concept is due to the spurious inclusion of the part y in the whole x. In the case of the shell weights under consideration this error is negligible, as is illustrated by the comparison of indices obtained in several groups of eggs as shown in table 3.

TABLE 3.—*Extent of error introduced in the ordinary allometric concept when utilized in determining relation between the weight of shell and egg weight for several samples of eggs*

Group No.	$\frac{\text{Weight of shell}}{\text{Weight of contents}^{2/3}}$	$\frac{\text{Weight of shell}}{\text{Weight of eggs}^{2/3}}$	Difference
TkG1	0.399	0.374	0.025
TkG2	.387	.362	.025
TkH1	.395	.367	.028
TkH2	.391	.365	.026
TkJ1	.398	.369	.029
TkJ2	.399	.373	.026
TkK1	.389	.363	.026
TkK2	.386	.363	.023

Although the values of the indices differ, the difference is consistent throughout, the deviations being maximized by the use of only three decimal places in the computations.

In general the advantage of these two methods over percentage of shell would lie in the fact that an exponential or logarithmic relation may logically exist between egg weight and shell weight, rather than a simple linear arithmetic one implying the identity of the limiting equilibrium constant α with unity. If in reality α differs greatly from unity, the correlation between egg weight and shell weight will be nonlinear and lower than the correlation between the logarithms of these variables. In an effort to learn whether or not the usage of percentage of shell in the statistical analyses proposed would lead to serious errors, comparable coefficients of correlation for several samples of eggs were computed, with the results shown in table 4. The spurious nature of the relation involved will be referred to later (p. 390).

For all practical purposes the two series of coefficients are identical. This would argue for the permissibility of using percentage of shell as

the measure of amount of shell. The particular utility of this, in addition to simplifying computation, lies in the fact that the program of selection of the thick and thin lines was based on percentage of shell rather than on any of the other measures. Furthermore, it enables the calculation of the spurious relation between the weight of the part (shell) and the weight of the whole (egg) by the method of Pearl (*13*) for index correlations.

TABLE 4.—*Comparable coefficients of correlation between egg weights and shell weight for several samples of eggs as calculated directly and by use of logarithms*

Group No.	Correlation coefficients between—	
	Egg weight and shell weight	Log egg weight and log shell weight
Tk1	0.762	0.750
Tk2	.700	.706
Tn1	.780	.771
Tn2	.754	.755

2. The rate of change in egg weight and shell weight from position 1 to position 2 in a clutch can be calculated either by ordinary percentage decrease or increase or on the basis of the continuous compound-interest formula $\log_n W_2 - \log_n W_1$. The latter procedure was adopted in view of the fact that successive negative increments in egg weight during a single clutch were found to decrease rather than to be maintained at the same level.

The data in table 5 indicate that the rate of decrease in egg weight or in its constituents is the function to be dealt with rather than the absolute decrease in relation to the original weight. Hence, in the calculation of the rate of change from position 1 to position 2, the difference between the natural logarithms of egg weight or of shell weight were used. The ratio of the latter over the former indicates the rate of decrease in shell weight relative to the rate of decrease in egg weight.

3. The coefficients of correlation were calculated in accordance with the method of Wallace and Snedecor (*17*), their table 16 being used for standards of significance.

4. Gross, spurious, and net coefficients of correlation where percentage of shell is involved were computed, as mentioned, by Pearl's method. The amount of spurious correlation when egg weight and shell weight were correlated was, however, calculated from the formula cited by Davenport and Ekas (*4, p. 90*).

5. Standard errors are used throughout in preference to probable errors.

TABLE 5.—*Rate of change in egg weight from position 1 to position 4 in a clutch*

Weight of first egg in clutch	Clutches	Decrease in weight between positions		
		1 and 2	2 and 3	3 and 4
Grams	*Number*	*Grams*	*Grams*	*Grams*
45.1–50.0	12	0.833	0.592	0.517
50.1–55.0	8	2.575	.625	−.475
55.1–60.0	11	2.109	.882	.009

EXPERIMENTAL RESULTS

STATISTICAL CONSTANTS FOR EGG WEIGHT AND SHELL CHARACTERS

The interrelations of four variables were investigated in this study: Egg weight, shell weight, shell thickness, and percentage of shell. Statistical constants for the variables are presented in table 6, showing the means with their respective standard errors and the coefficients of variation for the four generations of the thick and thin selections. A further subclassification of each generation is made on the basis of the position of the egg in the clutch. As will be shown later (p. 392), the thick and thin lines differ in their behavior with respect to the changes observed in the eggs laid in different positions in the cycle. Hence position 1 and position 2 of each clutch are treated here separately. A slight discrepancy in the number of birds involved will be noted between table 1 and table 6. This discrepancy occurs because the determinations of shell percentages on which the breeding selections were based were obtained irrespective of clutch position. Hence a few birds which laid in one-egg cycles or in very long cycles are included in table 1 but not in table 6. On the other hand a number of birds laid several two-egg clutches and are included in table 6, but not in table 1, because their total production in the period of the annual recording of data was less than 10 eggs, owing to pause, late maturity, or mortality. It was considered that no representative individual percentage of shell could be determined for such birds, yet the complete two-egg or larger clutches they produced could be included in the general population analysis. Because of the fact that the selected lines did not excel in production and because only a maximum of 10 determinations was made for each bird, only positions 1 and 2 in the clutch were adequately represented in the data.

Data on the coefficients of variation for egg weight and shell characters according to clutch position for the various generations of the two selected lines show that, except for egg weight in the thick-shelled line, no uniform trend of reduction in variability of these characters with successive generations of selection and inbreeding is apparent. This may be interpreted as indicating either that no pronounced increase in homozygosity was effected for these characters in either line during the generations of selection reported or that variability controlled by heredity in these characters is obscured by the variability normally found within the production of individual birds. Further, the two lines are quite similar in the degree of variability of the characters considered. In table 1 the coefficients of variation of the daughters' means with respect to percentage of shell are given. Here some reduction of variability is apparent between the first and second generations of both lines. In later generations variability increased slightly over that of the second generation. Table 7 presents an analysis of the significance of differences between the means of the thick- and thin-shelled lines for the characters reported in table 6. The two selected lines were significantly different beginning with the first generation for the characters of shell weight and percentage of shell. Except for shell thickness of eggs laid in position 1, G generation, the same was true for shell thickness. In the last three generations the egg weight also differed significantly between the lines.

131451—39——6

TABLE 6.—*Statistical constants for all eggs produced in first and second clutch positions by the thick- and thin-shelled lines*

Line and generation	Clutch position	Birds	Eggs	Means [1]				Coefficients of variation			
				Egg weight	Shell weight	Shell thickness	Percent shell	Egg weight	Shell weight	Shell thickness	Percent shell
		Number	*Number*	*Grams*	*Grams*	*Millimeters*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Thick:											
G	1	36	101	52.50±0.47	5.23±0.055	0.359±0.0029	9.96±0.062	8.93	10.52	8.08	6.22
	2	36	101	50.23± .46	4.92± .051	.348± .0025	9.79± .064	9.28	10.37	7.18	6.54
H	1	43	99	53.43± .40	5.21± .040	.368± .0023	9.76± .056	7.47	7.68	6.25	5.74
	2	43	99	50.68± .39	5.00± .038	.366± .0021	9.88± .058	7.60	7.60	5.74	5.88
J	1	30	79	52.75± .41	5.21± .048	.377± .0024	9.89± .072	6.90	8.25	5.57	6.48
	2	30	79	50.22± .36	5.07± .046	.376± .0028	10.10± .075	6.29	8.09	6.65	6.63
K	1	22	52	49.86± .43	4.94± .072	.368± .0049	9.90± .118	6.16	10.53	9.51	8.07
	2	22	52	48.19± .50	4.79± .064	.368± .0037	9.96± .104	7.41	9.60	7.34	7.55
Thin:											
G	1	5	12	50.94±1.01	4.82± .118	.346± .0061	9.46± .202	6.87	8.51	6.07	7.40
	2	5	12	49.17±1.02	4.59± .136	.328± .0055	9.34± .199	7.20	10.24	5.79	7.40
H	1	28	80	49.11± .37	4.67± .040	.347± .0022	9.51± .065	6.72	7.71	5.76	6.10
	2	28	80	47.39± .37	4.38± .045	.335± .0026	9.26± .075	7.07	9.13	6.87	7.25
J	1	20	58	50.63± .40	4.69± .046	.348± .0028	9.27± .075	5.96	7.46	6.03	6.16
	2	20	58	48.71± .37	4.52± .045	.347± .0026	9.30± .079	5.81	7.52	5.76	6.47
K	1	18	56	45.92± .51	4.32± .080	.346± .0036	9.39± .099	8.30	13.89	7.80	7.86
	2	18	56	44.60± .45	4.10± .055	.334± .0033	9.20± .082	7.58	10.00	7.49	6.64

[1] Standard errors, not probable errors, are used.

TABLE 7.—*Differences between thick- and thin-shelled lines for eggs produced in the first and second clutch positions*

[Thick line minus thin line]

Generation	Clutch position	Egg weight [1]	Shell weight [1]	Shell thickness [1]	Percent shell [1]
		Grams	*Grams*	*Millimeters*	*Percent*
G	1	[2] 1.56±1.11	0.41±0.130	[2] 0.013±0.0068	0.50±0.211
	2	[2] 1.06±1.12	.33± .145	.020± .0060	.45± .209
H	1	2.32± .55	.54± .057	.021± .0032	.25± .085
	2	3.29± .54	.62± .058	.031± .0033	.62± .095
J	1	2.12± .57	.52± .066	.029± .0037	.62± .104
	2	1.51± .52	.55± .064	.029± .0038	.80± .109
K	1	3.94± .67	.62± .108	.022± .0061	.51± .154
	2	3.50± .67	.69± .084	.034± .0050	.76± .132

[1] Standard errors, not probable errors, are used.
[2] Not significant.

These data show that, effective from the first generation of selection, significant differences in amount, thickness, and percentage of shell were obtained. Yet there is little evidence of any effect of the further selection of the type used in decreasing or increasing respectively the amount of shell, shell thickness, or percentage of shell in the two lines. The method of selection employed was successful in segregating thick- and thin-shelled lines, but not in modifying the expression of the shell characters within either line.

CORRELATION COEFFICIENTS BETWEEN EGG WEIGHT AND SHELL CHARACTERS

Table 8 presents coefficients of correlation between the different variables involved. Since shell weight is a part of egg weight and percentage of shell is an index of the two, gross correlations between these variables include a certain amount of spurious correlation. By subtracting the spurious part from the gross, the net coefficients of correlation are obtained. All of these appear in table 8 for each generation, each line, and each clutch position.

Though correlation coefficients in each column vary considerably in magnitude, general trends may be noted. With the exception of the

TABLE 8.—*Correlation coefficients between egg weight*

Line and generation	Clutch position	Eggs	Egg weight / Shell thickness	Shell weight / Shell thickness	Shell thickness / Percent shell
		Number			
Thick:					
G	1	101	0.327	0.721	0.745
	2	101	.203	.660	.778
H	1	99	.049	.583	.740
	2	99	.039	.603	.765
J	1	79	−.060	.628	.854
	2	79	.019	.740	.887
K	1	52	.291	.814	.855
	2	52	.123	.755	.823
Thin:					
G	1	12	−.052	.653	.849
	2	12	.067	.381	.468
H	1	80	.163	.761	.802
	2	80	.111	.750	.982
J	1	58	−.011	.723	.915
	2	58	−.071	.671	.844
K	1	56	.584	.834	.755
	2	56	.246	.761	.860

correlations between egg weight and shell thickness, most of the coefficients reported are statistically highly significant. The majority of exceptions to this lie in the G generation of the thin line, where only 12 pairs of eggs are represented. The correlations between shell weight and shell thickness (with the exception of the G generation, position 2, thin line) lie in the range between 0.583 and 0.834 and do not appear to depend on the origin of the sample. Similarly, the correlation coefficients between shell thickness and percentage of shell (with the same exception) range from 0.740 to 0.982 and are higher than those between shell weight and shell thickness in the previous column with the single exception of the K generation, thin line for position 1. Thus, while to a great degree, shell thickness is independent of egg weight, it seems to be adequately represented by percentage of shell and somewhat less so by the weight of shell.

The gross coefficients of correlation between egg weight and shell weight range between 0.558 and 0.820 and are again independent of the origin of the sample. These values are of the same general magnitude as those presented by previous investigators, such as 0.541 reported by Curtis (*3*), 0.644 by Jull (*8*), and 0.636 by Asmundson (*1*). The amount of spurious correlation is approximately 0.100 in all of the samples.

The index correlations between percentage of shell on the one hand and egg weight and shell weight on the other are of considerable interest. While the gross values do not show consistent significance, the elimination of the spurious correlations tends to change the picture. Thus percentage of shell and egg weight show in general a significant positive net correlation, varying in magnitude from 0.280 to 0.875. On the other hand the gross correlations between shell weight and percentage of shell are transformed from a low positive to a low negative net coefficient.

Evidently egg weight has a greater influence on the percentage of shell than has shell weight. While the increase in egg weight increases the percentage of shell, the increase in shell weight operates in the opposite direction. This, of course, applies to populations varying simultaneously in both components of the index, since obviously the reverse is true wherever one of the two components is constant.

and shell characters for thick- and thin-shelled lines

Egg weight / Shell weight			Egg weight / Percent shell			Shell weight / Percent shell			r at	
Gross	Spurious	Net	Gross	Spurious	Net	Gross	Spurious	Net	P=0.05	P=0.01
0.809	0.100	0.709	−0.068	−0.647	0.579	0.275	0.647	−0.372	0.195	.254
.745	.098	.647	−.219	−.667	.448	.334	.667	−.333	.195	.254
.707	.098	.609	−.351	−.697	.346	.377	.697	−.320	.195	.254
.713	.099	.614	−.379	−.707	.328	.379	.707	−.328	.195	.254
.647	.099	.548	−.241	−.641	.400	.376	.641	−.265	.217	.283
.599	.101	.498	−.217	−.805	.588	.380	.805	−.425	.217	.283
.665	.099	.566	−.106	−.689	.583	.261	.689	−.428	.273	.354
.619	.099	.520	−.191	−.611	.420	.368	.611	−.243	.273	.354
.605	.095	.510	−.246	−.628	.382	.388	.628	−.240	.576	.708
.663	.093	.570	−.054	−.575	.521	.316	.575	−.259	.576	.708
.663	.095	.568	−.268	−.548	.280	.378	.548	−.170	.217	.283
.669	.092	.577	−.140	−.612	.472	.341	.612	−.271	.217	.283
.616	.092	.524	−.226	−.624	.398	.379	.624	−.245	.250	.325
.558	.093	.465	−.250	−.612	.362	.398	.612	−.214	.250	.325
.820	.094	.726	.362	−.513	.875	.175	.513	−.338	.273	.354
.739	.092	.647	−.282	−.604	.322	.294	.604	−.310	.273	.354

EFFECT OF CLUTCH POSITION ON EGGSHELL CHARACTERS

A comparison of mean values for egg weight and shell weight for clutch positions 1 and 2 in both thin and thick lines (table 5) shows that, in every instance, the average eggs of position 2 are smaller and have less shell than those of position 1. The mean values for shell thickness and percentage of shell, however, do not have a similar uniform trend.

The relative percentage decreases in egg weight and shell weight from position 1 to position 2 and the ratio between them for each generation of each line are presented in table 9. In the G generation the percentage decrease of shell weight was greater than that of egg weight in both lines. In subsequent generations, however, the percentage decrease of shell weight in the thick-shelled line was markedly

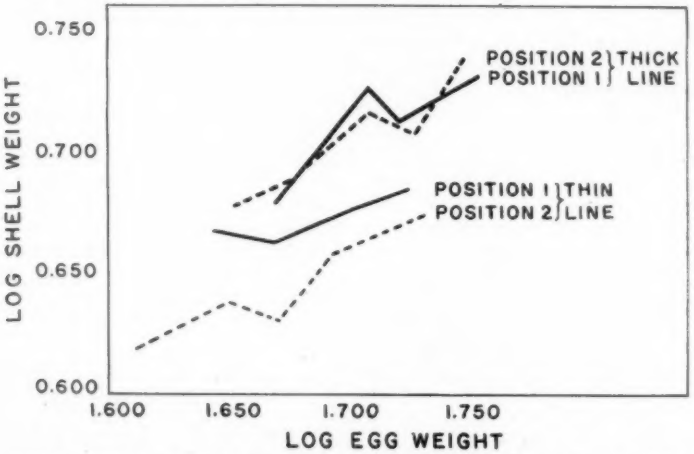


FIGURE 1.—Effect of clutch position on shell weight in relation to egg weight for the thick and thin lines in the H generation.

less than the corresponding decrease in egg weight. While considerable variation in the percentage decrease of both egg weight and shell weight took place in the thin-shelled line from generation to generation, in the last three generations the ratio of decrease of shell weight to that of egg weight was at least twice that observed in the thick-shelled line. In these generations the relative egg-weight decreases were greater in the thick-shelled than in the thin-shelled line; the reverse was true of relative shell-weight decreases.

The graphic presentation of the difference between the thick- and thin-shelled lines for the H generation shown in figure 1, indicates that eggs from clutch position 2 of the thin-shelled line have uniformly less shell than those from position 1 for the entire range of egg weight observed. On the other hand, in the thick-shelled line the eggs of clutch position 2 were not consistently different from those of position 1 with respect to shell weight in different egg-weight classes. Thus in the thick-shelled line the amount of shell relative to egg weight is maintained at approximately the same levels in positions 1 and 2, while in the thin-shelled line there is a pronounced decrease from position

1 to position 2. The K generation gives results similar to those of the H generation. In the J generation, however, the eggs of clutch position 2 in the thin-shelled line maintained an approximately equal relative decrease in egg weight and shell weight, while those from the thick shelled line had less relative decrease in shell weight and showed an increased percentage of shell (table 9).

TABLE 9.—*Rate of decrease* [1] *of egg weight and shell weight from position 1 to position 2 in a clutch for thick- and thin-shelled lines*

[Means of hen means with 3 or more clutches]

	Thick-shelled line				Thin-shelled line			
Generation	Hens	Egg weight [2]	Shell weight [2]	Ratio	Hens	Egg weight [2]	Shell weight [2]	Ratio
	Number	*Percent*	*Percent*		*Number*	*Percent*	*Percent*	
G	23	4.67±0.74	6.59±1.01	1.41	3	3.68±1.22	4.77±0.92	1.30
H	17	6.01± .74	4.08±1.04	.68	18	3.71± .62	6.29± .92	1.70
J	13	5.34± .51	2.56± .78	.48	14	3.43± .71	3.36±1.17	.98
K	7	4.37±1.38	2.21±2.62	.51	12	2.76± .67	3.73±1.22	1.35

[1] The formula $\log_n W_2 - \log_n W_1$ was used to calculate the rate of decrease.
[2] Standard errors, not probable errors, are used.

Although the above results indicate that the thick-shelled line was able to maintain the relative amount of shell in eggs of clutch position 2 better than the thin-shelled line, individual differences in the relative amount of shell in clutch positions 1 and 2 occurred in both lines in all generations. Obviously, the kind of selection practiced was only partially effective in differentiating lines varying in ability to form relatively as heavy shells for eggs of clutch position 2 as for position 1.

RESULTS FROM CROSSES BETWEEN LINES

Data for average percentage of shell are given in table 1 for reciprocal F_1 generations and for a backcross of F_1 females to thin-shelled-line males. Differences in percentage of shell for the K series between the various crosses and the selected lines are given in table 10.

The thick-shelled line is significantly higher in percentage of shell than the F_1 of thin female × thick male or of the backcross of F_1 $\frac{\text{thin } ♂}{\text{thick } ♀}$ female × thin male. The thick-shelled line is not significantly different in percentage of shell from the F_1 from thick female × thin male. The thin-shelled line, on the other hand, is significantly lower than all other matings.

There is a significant difference between the reciprocal F_1 generations, the thick female × thin male giving the higher percentage of shell. While the thick female × thin male F_1 generation was significantly higher than the backcross of F_1 $\frac{\text{thin } ♂}{\text{thick } ♀}$ female × thin male, the differences between the reciprocal F_1 generation and this backcross are not significant.

Unfortunately, for purposes of clear comparison, the F_1 thick female × thin male dams produced in the J generation and mated to thin-shelled-line males to give the backcross in the K generation had a lower average percentage of shell than the same F_1's produced in the K generation. This may have been a factor in producing the sig-

nificant difference between the F_1 thick female × thin male and the backcross F_1 $\frac{\text{thin}\,♂}{\text{thick}\,♀}$ female × thin male, as given in table 10. All the difference between these two generations cannot safely be attributed to the influence of the thin-shelled-line males used in the backcross.

TABLE 10.—*Differences in percentage of shell* [1] *for the K generation*

[Ordinates minus abcissae]

Item	Thick ♀ × thin ♂	$\frac{\text{Thin}\,♂}{\text{thick}\,♀}$ × thin ♂	Thin ♀ × thick ♂	Thin
Thick	[2]−0.12±.085	0.28±0.133	0.24±0.100	0.59±0.098
Thick ♀×thin ♂		.40± .123	.36± .086	.71± .085
Thin ♂ ♀×thin ♂			[2]−.04± .134	.31± .133
Thick ♀				
Thin ♀×thick ♂				.35± .100

[1] Standard errors, not probable errors, are used.
[2] Not significant.

Unfortunately in the J generation no offspring were secured from a attempted thin female mating × thick male; hence the reciprocal backcross in the K generation could not be made.

Because of the differences in egg weight between the thick- and thin-shelled lines (table 6), it may be suggested that the shell percentage depends on egg size not only within lines as shown by the net correlation coefficients in table 8, but also between lines. However, that such is not the case can be demonstrated:

(1) By figure 1, where percentage of shell differed between the two lines in eggs of the same weight, and (2) by the fact that, while the difference in percentage of shell between the reciprocal crosses was significant (table 10), the mean egg weights for the thin female × thick male and the thick female × thin male offspring were respectively 48.79±0.65 and 48.95±0.67 g.

DISCUSSION

A segregation of two lines of chickens differing significantly in amount, thickness, and percentage of eggshell based upon phenotypic selection of females and mating with related males was obtained in one generation and continued for three further generations. The evidence that the shell character of the dam was of extreme significance in determining that of the female offspring, as revealed by the results from reciprocal F_1 generations, explains why the two lines became significantly different so readily despite the relationship between sires used in establishing the two lines.

A genetic explanation of the above results cannot easily be deduced. Since eggshell characters are sex-limited in their expression, no phenotypic classification of the males, such as formed the basis of selection of the females, was possible. The comparative impotence of some of the thin-line males in affecting the shell characters of their daughters in the above experiment might indicate either (1) that the greater number of them were heterozygous for genes controlling the type of eggshell produced or (2) that they were less influential than the dams in determining the phenotype of the daughters. An interpretation of the results presented in table 10 on the basis of a maternal effect

would not be justified in the absence of experimental evidence disproving the first alternative suggested above.

A method of selection of breeding females which disregards the effect of differences in inherent rate of production on eggshell characters seems to be limited in its results. Although in this work it seemed to produce lines with significantly different percentages of shell, further generations of the same type of selection gave no greater differentiation between the lines or any apparent increase in homozygosity, which would be shown by decreased variability of the expression of shell character. Since the discovery of the differential effect of clutch position in the two lines, it is possible that two factors affect the shell percentage of an individual: (1) The amount of shell characteristically produced for the first egg of the clutch and (2) the ability of the individual to maintain the shell thickness and percentage in subsequently produced eggs of the clutch. If these are valid, separable factors, the failure to secure greater uniformity in the lines selected for four generations can be readily understood.

A selection of potential breeding females for relatively high percentages of eggshell during the winter months of the pullet year of production does seem to be practicable. In these months, when according to Baskett, Dryden, and Hale (*2*) a relatively uniform strength of shell is maintained, approximately 10 percent of the total egg weight as eggshell may be recommended as a desirable qualification for a breeding female.

The physiological implications of hereditary conditions affecting the metabolism and efficient utilization of calcium in the hen cannot be ignored (*12*). The meager evidence available on calcium balance in the laying hen (*10*) indicates that there are individual differences between birds in ability to maintain a positive calcium balance and an efficient output of calcium as eggshell. The possibility that hens which show a relatively high decrease in amount and proportion of eggshell with successive eggs of a clutch may be in some way inefficient in calcium metabolism should not be overlooked. The interrelation between hereditary, nutritional, and physiological factors influencing the character of eggshell needs to be determined.

SUMMARY

A selection of hens producing thin- and thick-shelled eggs, respectively, and a subsequent breeding program of mating with closely related males were effective in establishing two lines of chickens producing significantly different types of eggshell.

Continued selection of this type was ineffective in further differentiation of the two lines or in decreasing the variability of expression of eggshell characters in subsequent generations.

Analysis of the data indicated that heritable factors were involved in the determination of the amount, thickness, and percentage of eggshell characteristic of individual hens. In several matings the dams seemed to have relatively more influence in determining the character of shell produced by the daughters than the sires.

The females of the thick-shelled line maintained the ability to produce eggs with relatively little or no decrease in shell percentage from clutch positions 1 to 2. The pullets of the thin-shelled line, however, showed a greater tendency toward lower shell percentages in eggs in position 2. Individual variations in this respect were found in both lines.

The possible relation between hereditary factors and the nutritional requirements and physiological efficiency of laying hens with respect to calcium metabolism and balance is pointed out.

In the treatment of data on the populations available for this study, the percentage of total egg weight represented by eggshell was found to be as accurate an expression of individual variation as more cumbersome formulas which take into account the effect of differences in total egg weight upon shell weight.

LITERATURE CITED

(1) ASMUNDSON, V. S.
1931. THE FORMATION OF THE HEN'S EGG. Sci. Agr. 11: 590–606, 662–680, 775–788, illus.

(2) BASKETT, R. G., DRYDEN, W. H., and HALE, R. W.
1937. INVESTIGATION ON THE SHELL STRENGTH OF HEN EGGS. Jour. Min. Agr., North. Ireland 5: 132–142.

(3) CURTIS, MAYNIE R.
1914. A BIOMETRICAL STUDY OF EGG PRODUCTION IN THE DOMESTIC FOWL. IV. FACTORS INFLUENCING THE SIZE, SHAPE, AND PHYSICAL CONSTITUTION OF EGGS. Wilhelm Roux' Arch. Entwickl. Mech. Organ. 39: [217]–327, illus.

(4) DAVENPORT, C. B., and EKAS, MERLE P.
1936. STATISTICAL METHODS IN BIOLOGY, MEDICINE AND PSYCHOLOGY. Rev. ed. 4, 216 pp., illus. New York and London.

(5) HENDRICKS, WALTER A., LEE, ALFRED R., and GODFREY, ALBERT B.
1931. EFFECTS OF COD-LIVER OIL AND ULTRA-VIOLET IRRADIATION, AS INFLUENCED BY OYSTER SHELL, IN THE DIET OF CONFINED LAYING HENS. Jour. Agr. Research 43: 517–535, illus.

(6) HERRASTI, G.
1916. THE STRENGTH OF EGGSHELLS. (Letter to editor) Sci. Amer. 115: 321, illus.

(7) HUXLEY, J. S., and TEISSIER, G.
1936. TERMINOLOGY OF RELATIVE GROWTH. Nature [London] 137: 780.

(8) JULL, M. A.
1924. EGG WEIGHT IN RELATION TO PRODUCTION. PART I. THE RELATIONSHIP OF THE WEIGHTS OF THE PARTS OF THE EGG TO THE TOTAL EGG WEIGHT. Poultry Sci. 3: 77–88.

(9) MORGAN, C. L.
1932. RELATIONSHIP BETWEEN BREAKING STRENGTH AND THE PERCENT OF EGG SHELL. Poultry Sci. 11: 172–175.

(10) ——— and MITCHELL, J. H.
1938. THE CALCIUM AND PHOSPHORUS BALANCE OF LAYING HENS. Poultry Sci. 17: 99–104.

(11) ——— MITCHELL, J. H., and RODERICK, DONALD G.
1931. THE EFFECT OF COD LIVER OIL ON THE QUALITY OF EGG SHELLS. Poultry Sci., Poultry Sci. Assoc. Proc. (1930) 22: 39–41, illus.

(12) MUNRO, S. S.
1938. EFFECT OF HEREDITY ON INTERIOR EGG QUALITY AND SHELL COMPOSITION. Poultry Sci. 17: 17–27.

(13) PEARL, RAYMOND.
1907. A BIOMETRICAL STUDY OF CONJUGATION IN PARAMECIUM. Biometrika 5: [213]–297, illus.

(14) ROMANOFF, ALEXIS L.
1929. STUDY OF THE PHYSICAL PROPERTIES OF THE HEN'S EGGSHELL IN RELATION TO THE FUNCTION OF SHELL-SECRETORY GLANDS. Biol. Bull. 56: 351–356, illus.

(15) STEWART, GEORGE F.
1936. SHELL CHARACTERISTICS AND THEIR RELATIONSHIP TO THE BREAKING STRENGTH. Poultry Sci. 15: 119–124, illus.

(16) TAYLOR, LEWIS W., and MARTIN, J. HOLMES.
1928. FACTORS INFLUENCING THICKNESS OF EGGSHELL. Poultry Sci. 8: 39–44.

(17) WALLACE, H. A., and SNEDECOR, GEORGE W.
1931. CORRELATION AND MACHINE CALCULATION. Rev. by George W. Snedecor. Iowa State Col. Off. Pub. 30, No. 4, 71 pp., illus.

(18) WILLARD, J. T., and SHAW, R. H.
1909. ANALYSES OF EGGS. Kans. Agr. Expt. Sta. Bull. 159, pp. 143–177.

U. S. GOVERNMENT PRINTING OFFICE: 1939